Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

1,1-dichloroethylene; vinylidene chloride

EC Number: 200-864-0
CAS Number: 75-35-4

CLH-O-0000007324-77-01/F

Adopted

8 June 2023

8 June 2023

CLH-O-0000007324-77-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **1,1-dichloroethylene; vinylidene chloride**

EC Number: **200-864-0**

CAS Number: **75-35-4**

The proposal was submitted by **France** and received by RAC on **12 April 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/***on **14 June 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 August 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christina Tsitsimpikou advised by Nikolaos Spetseris**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2023** by **consensus**.

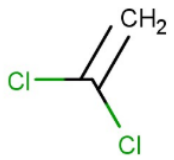
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	Flam. Liq. 1 Carc. 2 Acute Tox. 4*	H224 H351 H332	GHS02 GHS08 GHS07 Dgr	H224 H351 H332			D
Dossier submitters proposal	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	**Retain** Flam. Liq. 1 **Add** Muta. 2 Acute Tox. 3 STOT RE 1 Aquatic Chronic 3 **Modify** Carc. 1B Acute Tox. 1	**Retain** H224 **Add** H341 H301 H372 (liver, kidney, respiratory tract) H412 **Modify** H350 H330	**Retain** GHS02 GHS08 Dgr **Add** GHS06 **Remove** GHS07	**Retain** H224 **Add** H341 H301 H372 (liver, kidney, respiratory tract) H412 **Modify** H350 H330		**Add** inhalation: ATE = 0,5 mg/L (vapours) oral: ATE = 200 mg/kg bw	
RAC opinion	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	**Retain** Flam. Liq. 1 **Add** Muta. 2 Acute Tox. 3 STOT RE 1 Aquatic Chronic 3 **Modify** Carc. 1B Acute Tox. 1	**Retain** H224 **Add** H341 H301 H372 (respiratory tract, kidney, liver) H412 **Modify** H350 H330	**Retain** GHS02 GHS08 Dgr **Add** GHS06 **Remove** GHS07	**Retain** H224 **Add** H341 H301 H372 (respiratory tract, kidney, liver) H412 **Modify** H350 H330		**Add** inhalation: ATE = 0,5 mg/L (vapours) oral: ATE = 300 mg/kg bw	
Resulting Annex VI entry if agreed by COM	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	Flam. Liq. 1 Carc. 1B Muta. 2 Acute Tox. 1 Acute Tox. 3 STOT RE 1 Aquatic Chronic 3	H224 H350 H341 H330 H301 H372 (respiratory tract, kidney, liver) H412	GHS02 GHS08 GHS06 Dgr	H224 H350 H341 H330 H301 H372 (respiratory tract, kidney, liver) H412		inhalation: ATE = 0,5 mg/L (vapours) oral: ATE = 300 mg/kg bw	D

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

1,1-Dichloroethene, commonly called 1,1-dichloroethylene or vinylidene chloride (VDC), is a colourless liquid with a mild, sweet smell. It is an organochloride with the molecular formula $C_2H_2Cl_2$ and has the structure shown below. Like most chlorocarbons, VDC is poorly soluble in water, but soluble in organic solvents. In the absence of an added inhibitor, such as monomethyl ether of hydroquinone, vinylidene chloride readily polymerises. In the presence of air or oxygen, shock-sensitive and explosive peroxides are formed.



VDC is a man-made chemical that is not known to occur naturally. It is produced commercially via the dehydrochlorination of 1,1,2-trichloroethane in the presence of an aqueous alkali, like sodium hydroxide or lime. Vinylidene chloride can be purified through distillation and extraction.

VDC is used as an intermediate in organic synthesis reactions and in the production of a monomer in a variety of polymers. Several end products are made of VDC polymers, such as food plastic wrap, carpet latex backing, fire- and ignition-resistant clothing, vapour barriers for insulation, steel pipe coating, outdoor furniture, paper and board coatings, adhesives, and photographic film.

The substance is registered under the REACH Regulation and is manufactured in and/or imported to the European Economic Area at ≤ 1000 to < 10 000 tonnes per year.

VDC is restricted under the REACH regulation and listed in the Annex XVII (entry 38). The conditions of restrictions state that 1,1-Dichloroethylene "shall not be placed on the market, or used, as [a] substance or as constituent of other substances, or in mixtures in concentrations equal to or greater than 0,1 % by weight, where the substance or mixture is intended for supply to the general public and/or is intended for diffusive applications, such as in surface cleaning and cleaning of fabrics".

The current classification of VDC is published on the Adaptation to Technical Progress 00 (ATP 00) and originated from the previous European directive 67/548/CEE:

- Flam. Liq. 1 – H224
- Acute Tox. 4* - H332
- Carc. 2 – H351
- Note D: "Certain substances which are susceptible to spontaneous polymerisation or decomposition are generally placed on the market in a stabilised form….."

The current harmonised classification is based on data available at the time. Since then, new data has been generated which have been reviewed by the dossier submitter (DS). The main data sources for the CLH dossier as well as for the current RAC opinion are:

- NTP 2015
- IARC 2019
- Comet assay (Anonymous, 2016)

The DS, considering the new generated data as well as the self-classifications proposed in the C&L Inventory, justified that action is needed at a Community level and the following endpoints are assessed in the CLH report:

- Acute toxicity (oral and inhalation)
- Serious eye damage/eye irritation

- Germ cell mutagenicity
- Carcinogenicity
- Specific target organ toxicity — repeated exposure
- Short-term (acute) aquatic hazard
- Long-term (chronic) aquatic hazard

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of available information on toxicokinetics

The toxicokinetic data on VDC has been reviewed in the recent U.S. National Toxicology Program (NTP, 2015) and the International Agency for Research on Cancer (IARC, 2019) reports and was summarised by the DS.

Exposure to VDC predominantly takes place via the oral and inhalation route. The latter is regarded as the main one, since VDC is a very volatile liquid with a boiling point of 30.85°C at 1 atm and a high vapour pressure (66340 Pa at 20°C). Because of its low relative molecular mass and hydrophobic nature, dermal absorption is also likely, but there are no relevant published data. The chemistry of the VDC, its reaction products and metabolites as well as the toxicokinetic information about their distribution and/or persistency at specific sites/organs after exposure, are important aspects in the evaluation of the toxicity of VDC. Available data come mainly from studies on rodents.

Absorption

VDC is well absorbed from the lungs and gastrointestinal (GI) tract as it is a small, uncharged, lipophilic molecule. Following inhalation exposure in rats, the absorption of VDC was rapid and concentration dependent. The uptake was linear for concentrations up to 150 ppm, above which the uptake decreased with the increasing concentration. The compound was found in blood of rats within 2 minutes following exposure.

Following oral administration of doses ranging from 0.5 to 100 mg/kg bw, VDC was rapidly and almost completely absorbed in rats and mice and distributed to all tissues examined. Peak blood levels were observed in rats within 2 to 8 minutes.

The administration of equivalent oral and inhaled doses to rats results in significantly higher arterial blood levels and nephrotoxicity in animals inhaling the chemical.

Distribution

Although VDC is rapidly distributed to all tissues examined following either oral or inhalation exposure, most of the free VDC, its metabolites, and covalently bound derivatives are found in the liver and kidney. Following inhalation exposure to [^{14}C] VDC, the highest level of total radioactivity was found in the liver and kidney, with only small amounts present in other tissues. Covalently bound radioactivity was also highest in the liver and the kidney, where fasted rats presented higher levels than non-fasted. Mice were found to accumulate more VDC compared to rats (liver and kidney) under similar inhalation exposure conditions.

Following oral administration, VDC was rapidly and almost completely absorbed in rats and mice and distributed to all examined tissues with the highest amount found in the liver and kidney.

Metabolism

The proposed metabolic pathways of VDC in rodents are shown in the figure below. Cytochrome P450 (CYP) 2E1 is the predominant enzyme responsible for the oxidation of VDC. CYP2E1 metabolises VDC to three reactive electrophilic metabolites: vinylidene chloride epoxide (1), which is the major and likely the most important cytotoxic metabolite, 2-chloroacetyl chloride (2) and 2,2-dichloroacetaldehyde (3). These metabolites undergo oxidation, hydrolysis and reactions with glutathione (GSH) and other cellular macromolecules. Relatively high levels of CYP2E1 are present in rodents' liver, which is the most important site of VDC biotransformation, as well as in kidney and lung. Mechanistic studies using inducers and inhibitors of CYP2E1, or agents that deplete hepatic GSH levels, demonstrate the important role of biotransformation of VDC. The enzyme inducers enhance both the metabolic activation of VDC and cytotoxicity, while certain inhibitors decrease its biotransformation and toxicity. Detoxification of VDC by GSH is consistent with the observation that exposure to VDC depletes liver GSH levels. The highest CYP2E1 content is observed in centrilobular hepatocytes, followed by bronchiolar Clara cells and renal proximal tubular cells in the mouse.

In vitro studies have also shown that levels of acetal produced via metabolite (3) in lung microsomes were higher than those in the liver.

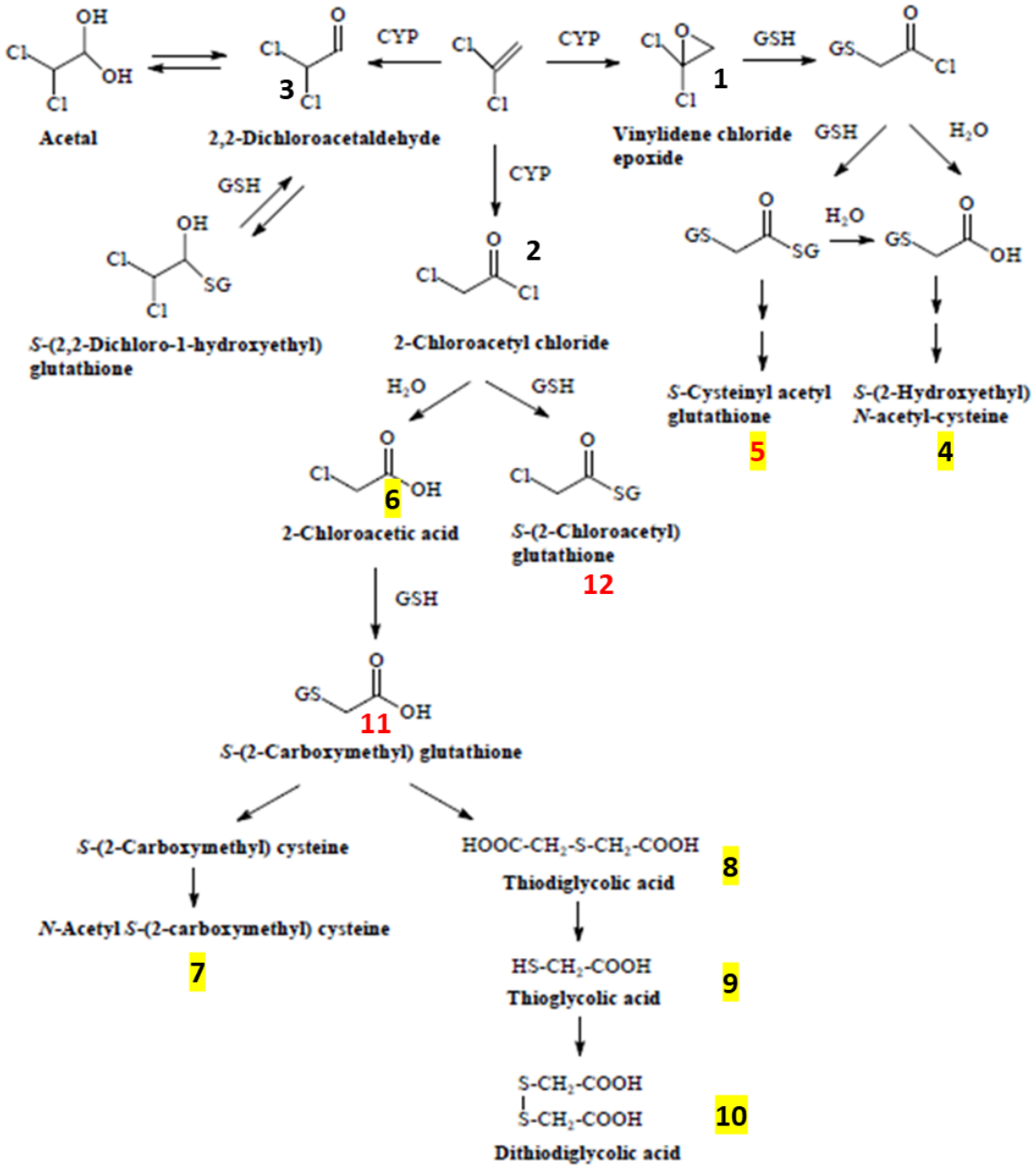


Figure: Proposed metabolic pathways of VDC in rodents (*in vivo* and *in vitro* data). Urine metabolites are highlighted with yellow colour; red colour represents biliary metabolites.

There are several critical factors that contribute to the metabolism of VDC. GSH levels and glutathione S-transferase activity, nutritional status (fasting and non-fasting) and changes in CYP2E1 are important parameters in the metabolic pathway of VDC. It has been shown that fasting (inducing GSH depletion) significantly reduces detoxification and enhances covalent binding of toxic metabolites in the liver and kidney. VDC metabolism occurs with different affinities and catalytic efficiencies in different species, suggesting species differences in the severities of toxicities by VDC. Humans appear to be less sensitive, as the mean rate of formation of the epoxide was two-fold higher in mouse lung and liver microsomal incubations compared to those from humans. In addition, renal CYP2E1 activity in humans is very low. The rate of formation of vinylidene chloride epoxide (1) and 2,2-dichloroacetaldehyde (3) was much lower in human lung and liver microsomes compared with that of mouse. Rats and mice present a similar metabolic profile qualitatively, but mice have increased metabolic output compared to rats regarding orally administered VDC. The formation of vinylidene chloride epoxide (1) is

expected to be higher in mice than in rats, since metabolite (4), which is a product of the reaction between metabolite (1) with GSH, is more abundant in mice than rats.

Sex differences have also been observed; VDC metabolism by kidney microsomes from male mice was six times greater than that from females. Sex differences were also observed in the CYP2E1-catalysed metabolic activation of VDC in mouse lung microsomes with the following order: adult female > weanling male = weanling female > adult male.

Elimination

The primary route for elimination of unchanged VDC is exhalation, while the primary route for elimination of metabolites is urinary excretion. Following inhalation exposure in rats, elimination of VDC was rapid, with most of the dose eliminated in the urine. Steady state levels in expired air were achieved following VDC exposure at 25 to 150 ppm with about 1% of the dose excreted unchanged in the expired air, indicating that the elimination is first order at these levels. At concentrations greater than 150 ppm, levels in expired air increased indicating saturation of metabolism. Pulmonary elimination was biphasic in rats after inhalation exposure, as a result of the lipophilicity of VDC. A 2-compartment model was followed, where the substance equilibrates between blood and the adipose tissue, for example. The half-lives for the first and second phases, respectively, based on the unchanged compound, were 20 and 217 minutes, respectively, following exposure to 10 ppm. When exposure to [^{14}C]VDC raised to 200 ppm, half-lives of 21 and 133 minutes were observed. Urinary elimination followed a similar pattern; the half-lives for the first and second phases, respectively, based on the total [^{14}C] excretion in urine, were 3.1 and 19.3 hours following exposure to 10 ppm and 3.8 and 23.9 hours following exposure to 200 ppm [^{14}C]VDC. During the rapid first phase, elimination mainly occurred via breathing and urine. Limited data in mice following inhalation exposure to 10 ppm VDC indicated that the elimination of unchanged compound in the expired air was smaller and elimination via urine was larger compared to rats, possibly due to a greater rate of metabolism in mice compared to rats.

Following oral administration, the pattern of elimination was similar to that following inhalation exposure. After a single administration of 1 mg/kg bw in rats, about 1% to 3% of the dose was excreted in expired air as parent compound. The majority of the dose was eliminated in urine (63%) and some in faeces (16%) within 72 hours, with the majority excreted within the first 24 hours. Following administration of 50 mg/kg bw, excretion of the parent compound in expired air increased to 16% - 30% of the dose, suggesting that metabolism saturates at rather low doses. Mice eliminated less in expired air as unchanged chemical and more in urine than rats following oral administration of 50 mg/kg bw in accordance with the inhalation results. The elimination, both pulmonary and urine, of VDC following oral administration in rats was also biphasic.

RAC evaluation of acute toxicity (oral and inhalation)

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS evaluated all six studies available for acute oral toxicity and in a weight of evidence approach proposed classification as Acute Tox. 3; H301: Toxic if swallowed with an acute toxicity estimate (ATE) of 200 mg/kg bw. The DS reached the conclusion based on two studies, the NTP (1982) (Klimisch 1) as the main study for classification and the Jones *et al.* (1978a) (Klimisch 4) as a supportive study also used for the determination of the ATE and by using the most sensitive species, the mouse.

No LD_{50} was calculated by the NTP, but according to the data, it should be between 50 mg/kg bw (1/10 animals died) and 500 mg/kg bw (8/10 animals died). The LD_{50} was estimated to be 365 mg/kg bw for females (maximum likelihood method). No LD_{50} could be calculated for males as no mortality was found at 100 mg/kg bw and 100% mortality occurred at the higher dose of 500 mg/kg bw. Although the LD_{50} from the NTP study was considered to warrant classification in Category 4, the DS argued that based on a precautionary principle and considering 1) the heterogeneity of results, 2) the date of the key study, 3) the fact that no LD_{50} was derived, and 4) the overall robustness of the database, a classification in Category 3 (50 mg/kg bw < ATE ≤ 300 mg/kg bw) was justified. This proposal was also supported by the results from the Jones *et al.* (1978a) study, which concluded an LD_{50} of 217 mg/kg bw for male mice and 194 mg/kg bw for female mice (without further information). The proposed LD_{50} of 200 mg/kg bw was rounded from the results of the Jones *et al.* (1978a) study.

Acute inhalation toxicity

The DS evaluated nine available studies and proposed classification as Acute Tox. 1; H330: Toxic if inhaled with an ATE of 0.5 mg/L based on the most sensitive species, the male mouse (Anonymous, 1979d).

In order to compare the sensitivity of 3 species to VDC (rats, mice and hamsters) and the effect of diet on the toxicity of the substance, the DS relied on Anonymous (1979a; 1979b; 1979c; 1979d; 1979e; 1979f). LC_{50} values were derived in two studies (Anonymous, 1979c, d) on fed and fasted mice: fasted males and females: 0.2 and 0.5 mg/L, respectively; fed males and females: 0.46 and 0.82 mg/L, respectively. Males were found consistently more sensitive than females after an acute inhalation exposure and therefore, classification in Category 1 was proposed by the DS with an ATE rounded to 0.5 mg/L based on the LC_{50} of fed male mice.

Comments received during consultation

Acute oral toxicity

There were two comments submitted during the consultation, one by a Member State Competent Authority (MSCA) and one by an Industry/Trade Association.

The MSCA questioned the proposed category of classification, suggesting Category 4 based on the LD_{50} value of 365 mg/kg bw (female mice) derived from the most reliable NTP study (1982). In addition, even in the case of a Category 3 classification, the MSCA argued that the converted acute toxicity point estimate (cATpE) of 100 mg/kg bw was unrealistic, and an ATE of 200 mg/kg bw could be proposed based on the worst-case scenario of the Jones *et al*. (1978a) study.

The DS replied that the NTP study, despite its high quality did not meet the current guidelines as it was not conducted to determine an LD_{50}. The value of 365 mg/kg bw, which was close enough to the upper ATE limit of Category 3, corresponded to female mice, which were expected to be less sensitive than male mice, based on the toxicological profile of VDC. Therefore, a lower LD_{50} was expected, pointing to a Category 3 classification, in line with the results of the other less reliable (lower Klimisch score) available studies.

In the second comment, Industry/Trade Association proposed that based on metabolic differences between different species, the toxicity observed in rats was the most representative of the toxicity expected in humans, and the acute classification should be based on the LD_{50} from rat studies. The lowest LD_{50} value observed in rats was 1500 mg/kg bw in female rats (Ponomarkov *et al*. 1980). Based on this value, Industry/Trade Association considered that VDC should be classified in Category 4; H302: Harmful if swallowed.

The DS responded that, in the absence of robust data, the Guidance on the Application of the CLP Criteria (CLP guidance) recommends using the most sensitive species, which is the reason why data on mice were used.

Acute inhalation toxicity

There were two comments regarding acute inhalation toxicity, one from a MSCA and one from Industry/Trade Association.

The MSCA supported the proposed classification as Acute Tox. 1; H330 with an ATE of 0.5 mg/L.

Similarly to the comment on acute oral toxicity, the Industry/Trade Association proposed that based on the metabolism differences between the species, the toxicity observed in rats was the most representative of the toxicity expected in humans, and the acute inhalation classification should be based on the LC_{50} from rat studies. For acute inhalation toxicity, 6 studies conducted in rats were available. The lowest LC_{50} reported for rats was 28.350 mg/L/4 h (Anonymous, 1979a), which did not warrant classification. However, there was a harmonised classification for VDC in Annex VI of CLP as Acute Tox. 4; H332. The Industry/Trade Association proposed to maintain this classification as a conservative approach.

The DS responded in the same manner as in the acute oral toxicity section.

Assessment and comparison with the classification criteria

Acute oral toxicity

Table: *Summary of acute oral toxicity studies*

Method, guideline, duration of exposure deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels**	**Value LD_{50} Classification**	**Reference**
Gavage Vehicle: corn oil Observation period: 14 days	Mice B6C3F1, male and female 5 animals/sex/dose	Vinylidene chloride Purity: 99 %	0, 10, 50, 100, 500 and 1000 mg/kg bw	50 mg/kg bw < LD_{50} < 500 mg/kg bw **Acute Tox. 3 or 4**	NTP, 1982 Klimisch 1 Key study
	Rat Fischer 344, male and female 5 animals/sex/dose			LD_{50} > 1000 mg/kg bw **Acute Tox. 4 or no classification**	
Gavage	Swiss OF, mice (IFFA-CREDO), Males (10/group)	Vinylidene chloride	200 mg/kg bw	LD_{50} > 200 mg/kg bw **Acute Tox. 3 or 4 or no classification**	Ban *et al*., 1995
Gavage Vehicle: corn oil	Holtzman male rats Number of animals/group not provided	Vinylidene chloride Purity not provided	Not specified	LD_{50} = 1510 mg/kg bw **Acute Tox. 4**	Jenkins *et al*., 1972 Klimisch 3
Gavage Vehicle: olive oil	Inbred BDIV rats	Vinylidene chloride	Not specified	LD_{50} = 1800 mg/kg bw for	Ponomarkov *et al*., 1980

	4 animals/sex/dose	Purity: 99 %		males, 1500 mg/kg bw for females. **Acute Tox. 4**	Klimisch 3
Intragastric Vehicle: corn oil	Alderley Park male and female mice 6 animals/sex/dose	Vinylidene chloride Purity not provided	5 groups, doses not specified	LD_{50} male = 217 mg/kg bw LD50 female = 194 mg/kg bw **Acute Tox. 3**	Jones *et al*., 1978a Klimisch 4
Oral, not further specified	Rat, strain and sex not specified	Vinylidene chloride Purity not provided	Not specified	LD_{50} = 2500 mg/kg bw **No classification**	Kennedy *et al*., 1991 Klimisch 4

The six available studies for acute oral toxicity are shown in the table above. Although most of these studies are old, not conducted according to current guidelines and lack proper reporting, they are published in well-respected peer-reviewed journals.

It is apparent from the table above that the most sensitive species is the mouse, with LD_{50} values ranging from 194 to 365 mg/kg bw for the most sensitive sex in each study. For rats, the respective LD_{50} values are > 1000 mg/kg bw. Classification should be based on mice because according to the CLP guidance (version 5, 2017): "*In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification*".

The most reliable study is the NTP (1982) study which, however, was a single range-finding study conducted as a preliminary assessment of toxicity aiming at studying the carcinogenic potency of VDC. Therefore, the authors did not derive an LD_{50}. The study was conducted both in mice and rats. The conditions of the study are listed in the table above and the mortalities observed in the table below.

Table: *Mortality data in mice and rats (NTP, 1982)*

Dose mg/kg bw	**Mice** #mortalities/#animals		**Rats** #mortalities/#animals	
	Male	Female	Male	Female
0	0/5	0/5	0/5	0/5
10	0/5	0/5	1/5	0/5
50	0/5	1/5	0/5	0/5
100	0/5	0/5	0/5	0/5
500	5/5	3/5	0/5	1/5
1000	5/5	5/5	2/5	0/5

For mice, the LD_{50} lies between 50 mg/kg bw (males: no lethality, females: 20 % lethality) and 500 mg/kg bw (males: 100% lethality, females: 60% lethality).

In accordance with the OECD TG 425, an LD_{50} of 365 mg/kg bw was estimated by the DS (Acute Tox. 4) using the maximum likelihood method for female mice. No LD_{50} can be calculated for males as no mortality was seen at 100 mg/kg bw and 100% mortality was observed at 500 mg/kg bw.

In the Ban *et al.* (1995) study, an LD_{50} could not be derived, but it is expected to be > 200 mg/kg bw, since no mortalities were observed in mice at 200 mg/kg bw with a monitoring period of 8

hours. It should be also noted that the study was not conducted as an acute oral toxicity study but rather to examine the mechanism of nephrotoxicity.

In the Jones *et al*. (1978a) study, published in the Br. J. Cancer, groups of mice were given a single oral dose of the test substance by gavage in corn oil at 5 dose levels. The LD_{50} values were calculated by using the Thompson's (1947) method of moving averages and interpolation both for male and female mice. No information on the control group, on the tested dose levels or on the post exposure was provided. At the time of this study, no guideline was available either for the method used or for the GLP. The LD_{50} values reported were 217 mg/kg bw for male mice and 194 mg/kg bw (within guidance value range for Acute Tox. 3) for female mice with no other information (including data on mortality rates).

In addition to the Jones *et al*. (1978a) study, in the micronucleus test in mice by Sawada *et al*. (1987), although not conducted for acute oral toxicity purposes, a 50 % mortality in male mice was observed at a single dose of 200 mg/kg bw, which could further support the proposed ATE.

The results of the available studies point to classification as acute oral toxicity, either Category 3 or Category 4.

RAC agrees with the DS that the results of the NTP study on mice should be used for classification purposes, with the Jones *et al*. (1978a) study as supporting evidence. The value of LD_{50} 365 mg/kg bw for female mice, as estimated by the DS using the maximum likelihood method, warrants classification in Category 4, but is close to the threshold value of ATE 300 mg/kg bw for Category 3 (22% higher). The Jones *et al*. (1978a) study supports Category 3 with an LD_{50}=194 mg/kg bw for female mice. These findings would lead to a borderline case between Category 3 (Jones *et al.,* 1978a) and 4 (NTP, 1982). In a weight of evidence approach, also taking into consideration the 50% mortality at 200 mg/kg bw observed in the Sawada *et al*. (1987) micronucleus study, RAC considers that classification as Acute Tox. 3 is justified.

RAC finds the cATpE for Category 3 (100 mg/kg bw), not to be realistic and the ATE of 200 mg/kg bw proposed by the DS too conservative, as explained above. Therefore, the upper ATE limit of Category 3, ATE of 300 mg/kg bw, is proposed.

In conclusion, RAC considers that classification of VDC as **Acute Tox. 3; H301: Toxic if swallowed** with an **ATE of 300 mg/kg bw** is warranted.

Acute inhalation toxicity

Table: *Summary of acute inhalation toxicity studies*

Method, guideline, duration of exposure deviations if any	**Species, strain, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels**	**LC_{50}**	**Reference**
Whole body vapour 4-h exposure 14-day observation period	Sprague-Dawley rat 10/sex/dose	Vinylidene chloride Purity: 99.7%	7.94, 19.84, 35.71, 59.52 mg/L	LC_{50} for males and females: **28.35 and 40.78 mg/L,** respectively. No classification	Anonymous, 1979a Klimisch 2
Whole body vapour 4-h exposure	Sprague-Dawley rat, **fasted** 10/sex/dose	Vinylidene chloride Purity: 99.7%	0.4, 1, 2, 4, 6, 8, 20, 40, 48, mg/L	LC_{50} for fasted males and females:	Anonymous, 1979b Klimisch 2

Method, guideline, duration of exposure deviations if any	Species, strain, no/group	Test substance, form and particle size (MMAD)	Dose levels	LC_{50}	Reference
14-day observation period				**1.63 and 26 mg/L,** respectively. Acute Tox. 2 or No classification	
Whole body vapour 4-h exposure 14-day observation period	Male Sprague-Dawley rat 16/dose	Vinylidene chloride Purity not provided	4900, 6150 ppm Corresponding to 19.6, 24.6 mg/L	LC_{50} = **25.4 mg/L** No classification	Siegel, 1971 Klimisch 3
Whole body vapour 4-h exposure 24-h observation period	Male Holtzman rats, fasted or fed 5-10/dose	Vinylidene chloride Purity not provided	0.2 to 80 mg/L	Estimated LC_{50}: Fed rats = **60 mg/L** Fasted rats = **2.4 mg/L** Acute Tox. 3 or no classification	Jaeger, 1974 Klimisch 3
Whole body vapour 22-23 h/d; 3-day exposure	CD rat 10/sex/dose	Vinylidene chloride Purity: 99%	0.060, 0.12 and 0.24 mg/L	No LD_{50}, no mortality	Short, 1977a Klimisch 3
Whole body vapour 22-23 h/d; 1-day exposure	CD-1 mouse 10/sex/dose	Vinylidene chloride Purity: 99%	0.060, 0.12 and 0.24 mg/L	LC_{50} (males, 22-23 h) = **0.39 mg/L (extrapolated to a 4-h exposure using Haber's law: 2.34 mg/L)** LC_{50} (females, 22-23 h) = **0.42 mg/L (extrapolated to a 4-h exposure using Haber's law: 2.52 mg/L)** Acute Tox. 3	Short, 1977a Klimisch 3
Whole body vapour 4-h exposure 14-day observation period	NMRI mouse, fasted 10/sex/dose	Vinylidene chloride Purity: 99.7%	0.04, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/L	LC_{50} for fasted males and females**: 0.2 and 0.5 mg/L,** respectively. Acute Tox. 1	Anonymous, 1979c Klimisch 2

Method, guideline, duration of exposure deviations if any	Species, strain, no/group	Test substance, form and particle size (MMAD)	Dose levels	LC_{50}	Reference
Whole body vapour 4-h exposure 14-day observation period	NMRI mouse	Vinylidene chloride No information on purity	No information	LC_{50} for males and females**:** **0.46 and 0.82 mg/L,** respectively. Acute Tox. 1 or 2	Anonymous, 1979d Klimisch 4
Whole body vapour 4-h exposure 14-day observation period	Chinese hamster, fasted 10-20/sex/dose	Vinylidene chloride Purity: 99.7%	0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L	LC_{50} for fasted males and females: **0.6 and 1.8 mg/L,** respectively. Acute Tox. 2	Anonymous, 1979e Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	Chinese hamster 10/sex/dose	Vinylidene chloride Purity: 99.7%	1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L	LC_{50} for males and females: 6.59 and 11.69 mg/L, respectively. Acute Tox. 3 or 4	Anonymous, 1979f Klimisch 2

All ten studies evaluated in the CLH report have deficiencies and were not performed according to OECD test guidelines. However, there is a series of studies by Anonymous (1979a-1979f), which aimed to assess acute inhalation toxicity of VDC in rats, hamsters and mice and to evaluate the effect of the animals' nutritional status (fasting) on inhalation toxicity. These studies were conducted according to a protocol similar to the current guidelines and they were considered as more reliable by the DS. RAC, in agreement with the DS, bases the classification for acute inhalation toxicity on these studies by Anonymous.

In these studies, 10 animals/sex/dose were whole-body exposed to VDC for 4 hours, with a post-exposure observation period of 14 days. It is noted that there are reporting deficiencies.

A comparison of the estimated LC_{50} values from the Anonymous (1979a-1979f) studies is summarised in the table below.

Table: *Comparison of LC_{50} values from the Anonymous (1979a-1979f) studies*

Method, guideline, duration of exposure	Species, strain, nutritional status, no/group	Dose levels	LC_{50} Male	LC_{50} Female	Reference
Whole body vapour 4-h exposure 14-day observation period	Sprague-Dawley rat **non-fasted** 10/sex/dose	7.94, 19.84, 35.71, 59.52 mg/L	**28.35 mg/L** No classification	**40.78 mg/L** No classification	Anonymous, 1979a Klimisch 2
	Sprague-Dawley rat, **fasted** 10/sex/dose	0.4, 1, 2, 4, 6, 8, 20, 40, 48, mg/L	**1.63 mg/L** Acute Tox. 2	**26 mg/L** No classification	Anonymous, 1979b Klimisch 2

Whole body vapour 4-h exposure 14-day observation period	Chinese hamster **non-fasted** 10/sex/dose	1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L	**6.59 mg/L** Acute Tox. 3	**11.69 mg/L** Acute Tox. 4	Anonymous, 1979f Klimisch 2
	Chinese hamster, **fasted** 10-20/sex/dose	0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L	**0.6 mg/L** Acute Tox. 2	**1.8 mg/L** Acute Tox. 2	Anonymous, 1979e Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	NMRI mouse **non-fasted** 10/sex/dose	No information	**0.46 mg/L** Acute Tox. 1	**0.82 mg/L** Acute Tox. 2	Anonymous, 1979d Klimisch 4 (due to reporting deficiencies)
	NMRI mouse, **fasted** 10/sex/dose	0.04, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/L	**0.2 mg/L** Acute Tox. 1	**0.5 mg/L** Acute Tox. 1	Anonymous, 1979c Klimisch 2

The following conclusions can be drawn from the data shown in the table above:

- mice are more sensitive than hamsters, which are more sensitive than rats;
- regardless of the species, males are consistently more sensitive to VDC than females;
- effects of VDC are exacerbated in fasted animals

It should be noted that the LC_{50} values discussed are those provided by the authors of the studies without any details on the calculation method used. Moreover, for the Anonymous (1979d) study, the data on mortalities per dose are not available. In addition, in the Anonymous (1979c) study, mortality reporting between consecutive doses seems unusual: 3/10 male died at 0.2 mg/L and 10/10 at 0.3 mg/L; 0/10 females died at 0.4 mg/L and 10/10 at 0.5 mg/L).

In rats the LC_{50} ranged from 1.63 mg/L (for fasted male rats) to 40.78 mg/L (for non-fasted female rats). Hamsters were more sensitive to the toxicity of VDC, with estimated LC_{50} varying according to sex and diet status from 0.6 mg/L (for fasted male hamsters) to 11.59 mg/L (for non-fasted females).

Lastly, in accordance with the results from the oral studies, the mouse proved to be the most sensitive species, with estimated LC_{50} values ranging from 0.2 mg/L for fasted males to 0.82 mg/L for non-fasted females.

Regarding classification, according to the CLP guidance (version 5, 2017) *"In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification".* Consequently, classification should be based on the results of the mouse studies, as the most sensitive species and the findings of the Anonymous (1979a-f) (Klimisch 2) studies, as the most reliable ones.

Since the nutritional status of fasting is known to affect the metabolism of VDC negatively and it is also not a condition/parameter of current testing guidelines, RAC agrees with the DS to use data on non-fasted male mice (Anonymous, 1979d). All relevant studies in male mice lead to a classification for acute inhalation toxicity, Category 1. Concerning the acute toxicity estimate (ATE), RAC agrees with the DS that the value of the cATpE (0.05 mg/L) appears not realistic as it is considered too low in comparison to the results of the two Anonymous (1979c, d) mouse

studies. Therefore, RAC supports the proposed rounded ATE of 0.5 mg/L based on the data from Anonymous (1979d).

In conclusion, RAC agrees with the proposal by the DS that classification of VDC as **Acute Tox. 1; H330: Toxic if inhaled with an LC_{50} of 0.5 mg/L (vapours**) is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS evaluated the two available studies for the classification of serious eye damage/eye irritation.

The first study (Anonymous, 1979g), although of low reliability, showed slight redness and slight oedema but the effects were reversible after 1 week.

The second study was an *in vitro* test on fresh bovine cornea, complying with the current GLP and OECD test guideline 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) requirements (Anonymous, 2010). The calculated mean *in vitro* score, mean IVIS = 43.90 of the study does not warrant a classification in Category 1. The OECD TG 437 for BCOP assay specifies that if the IVIS score is between 3 and 55, no stand-alone prediction can be made. Since this was the only available reliable study, the DS proposed no classification for VDC for serious eye damage/eye irritation due to inconclusive data.

Comments received during consultation

There was one comment by a MSCA that based on the same reasoning as the DS, supported the proposed no classification, as no further *in vitro* tests were available, and "no classification due to insufficient data".

Assessment and comparison with the classification criteria

The data for the two available studies, *in vivo* and *in vitro* (BCOP), respectively, are shown in the table below.

***Table**: Summary table of available animal studies on serious eye damage/eye irritation*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results - Observations and time point of onset - Mean scores/animal - Reversibility**	**Reference**
No standard method No guideline One eye of each rabbit treated with 50 µL of undiluted VDC solution and not washed. Same dose of physiological solution applied in	Vienna white rabbits 2 animals	vinylidene chloride Purity: 99.7%	50 µL undiluted No washing	1h after treatment: slight redness and slight edema. 24h after application: slight redness. 1 week after treatment: no significant effect.	Anonymous, 1979g Klimisch 3

the non-treated eye as control. Post exposure observation time points: 1h, 24h and 8 days					

Table: *Summary table of available in vitro studies for serious eye damage/eye irritation*

Guideline Type of study	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Guideline: yes OECD TG 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) GLP: yes	vinylidene chloride purity > 99%	VDC tested undiluted. Cornea opacity measured using the OP-KiT opacitometer. Permeability of the cornea possibly caused by the test item, measured at 490 nm (OD490) with a spectrophotometer.	Mean IVIS = 43.90.	Anonymous, 2010 Klimisch 1

The *in vivo* study did not follow current test guidelines and was considered as Klimisch 3 by the DS. The study was conducted on 2 Vienna white rabbits with undiluted VDC and without washing of the eye. The same dose of physiological solution was applied in the non-treated eye as control. The post exposure observation period was up to 8 days, with observation time points at 1h, 24h and 8 days after treatment. Individual detailed results were not available. Nonetheless, an hour after exposure slight redness and slight edema were observed in both animals. After one week, no significant effect was noted except a slight irritation of the lining of the eye thus rendering the effects reversible.

The *in vitro* test was performed according to OECD TG 437 (BCOP - Bovine Corneal Opacity and Permeability test method or BCOP assay) and to GLP and therefore, was considered as Klimisch 1 by the DS.

The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. In this test method, damage by the test chemical is assessed by quantitative measurements of changes in corneal opacity and permeability with an opacitometer and a visible light spectrophotometer, respectively. The BCOP test method uses isolated corneas from the eyes of freshly slaughtered cattle (calf or bovine). Corneal opacity is measured quantitatively as the amount of light transmission through the cornea. Permeability is measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea, as detected in the medium in the posterior chamber. The mean opacity and permeability values for each treatment group are combined in an empirically-derived formula to calculate an *in vitro* irritancy score (IVIS).

IVIS = mean opacity value (OP-KIT) + (15 x mean permeability OD490)

Based on the IVIS score of the BCOP test method, a chemical can be either classified as serious eye damage/eye irritation, Category 1 (IVIS > 55) or not classified at all for serious eye damage/eye irritation (IVIS ≤ 3). No conclusion for classification can be made based on IVIS

score of the BCOP 3 < IVIS ≤ 55 and further testing using *in vivo* methods is required. According to the CLP guidance (2017), a substance can be considered as causing serious eye damage (Category 1) based on positive results in the BCOP test. However, according to the CLP guidance, there are no *in vitro* tests with regulatory acceptance for eye irritation at present.

In the Anonymous (2010) study, VDC was tested undiluted, and three corneas were used in each group (test item, negative control and positive control). The negative control was a 0.9% NaCl solution and the positive control was 2-Ethoxyethanol.

The cornea opacity was measured using the OP-KiT opacitometer. In the second step of the assay, permeability of the cornea, as possibly caused by the test item, was measured at 490 nm (OD490) with a spectrophotometer. The results are shown in the Table below.

Table: *Results of VDC tested in BCOP assay after 10 minutes incubation time*

Test Group	Opacity Value		Permeability		In vitro score	IVIS
Negative Control	-1	Mean 0	0.052	Mean 0.055	-0.22	Mean 0.82
	0		0.051		0.77	
	1		0.061		1.92	
Positive Control	66.00	67.67	0.640	0.768	75.61	79.20
	68.00		0.874		81.12	
	69.00		0.791		80.87	
VDC	10.00	9.00	2.312	2.326	44.69	43.90
	9.00		2.306		43.60	
	8.00		2.360		43.41	

The calculated mean *in vitro* irritancy score (IVIS) for VDC is:

IVIS = 9 + 15 X 2.326 = 43.90

Based on this IVIS score, VDC cannot be considered as meeting the criteria for serious eye damage and therefore does not warrant classification in Category 1. No other stand-alone prediction can be made as explained above.

In conclusion, the results from Anonymous (1979g) study are not suitable for classification purposes and the results from the Anonymous (2010) study exclude classification for serious eye damage/(Category 1), but are not sufficient to draw a conclusion on no classification for eye irritation (Category 2), as the irritant potency of VDC cannot either be excluded or verified with the BCOP assay.

Therefore, RAC proposes **no classification of VDC for serious eye damage/eye irritation due to inconclusive data.**

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Studies both via the oral and inhalation route were assessed by the DS for STOT RE.

The DS evaluated the 13-week and 104-week NTP (1982) oral (gavage) studies in rats and mice and the Quast *et al*. (1983) studies in rats (2-year administration in drinking water) and dogs

(97-day administration in gelatine capsules). In all studies, liver was recognised as the target organ with various adverse effects of diverse severity (from changes in transaminases in dogs and cellular atypia in mice to centrilobular necrosis in rats). Chronic inflammation of the kidney was also reported in the 13-week NTP (1982) gavage study in rats. No exposure-related histopathological lesions were observed in dogs.

Studies in rats and mice showed relevant adverse effects within the CLP Regulation guidance value range for Category 2: 10 < C ≤ 100 mg/kg bw/day for a 90-day oral study.

By inhalation route, many more studies were available in various species, but only the recent NTP studies (NTP, 2015) were considered by the DS as of high quality (two-week and 13-week studies in rats and mice) and used as key studies to justify the classification proposal for VDC. In rats, adverse effects were described in the liver, characterised by centrilobular cytoplasmic alteration from minimal to moderate severity both in the two-week and in the 13-week study. Also effects in the olfactory epithelium were reported in the 13-week study at all concentrations (from 0.025 mg/L) tested. The severity of these effects was minimal at low doses and increased with increased concentrations. Effects in rat kidneys were also observed (renal tubules casts) at higher doses. In mice, effects were consistently observed in kidney, liver and nose (necrosis of the respiratory epithelium or respiratory epithelium squamous metaplasia).

The LOAEC associated to these critical effects were 0.025 mg/L in the 13-week rat study and 0.05 mg/L in the 13-week mouse study. After comparing the doses causing these effects with the CLP Regulation guidance values via the inhalation route, these 13-week studies were considered to fulfil the classification criteria for Category 1.

According to the CLP guidance (version 5, 2017), the final classification category for STOT RE based on non-human data should be the most severe classification category warranted by any of the three routes of exposure. Thus, the DS proposed a classification as STOT RE 1; H372: Causes damage to organs through prolonged or repeated exposure (liver, kidney, respiratory tract).

Comments received during consultation

During the consultation, an MSCA supported the proposed classification and the reasoning by the DS.

An Industry/Trade association disputed the proposed classification for STOT RE, as the lesions and histopathological findings used by the DS as the justification for STOT RE were considered as part of the carcinogenic activity of VDC.

The DS replied that tumours were to be assessed under carcinogenicity and had not been taken into consideration for the STOT RE classification. On the contrary, the effects such as hepatic centrilobular alteration, olfactory epithelium necrosis or even nephropathy were to be considered for classification purposes under STOT RE. Even if these effects were observed in the same organs recognised as target organs for carcinogenicity, they would not necessarily lead to the development of tumours.

Assessment and comparison with the classification criteria

Studies described in in Tables 22 and 23 of the CLH report are discussed hereafter.

Studies in rodents available by oral route (Table 22 of the CLH report) are of relatively good quality, all rated as Klimisch 2 studies. In all studies, liver was recognised as the target organ with various adverse effects observed, namely necrosis, hepatocytomegaly, portal and

subcapsular fibrosis, bile duct hyperplasia, hepatocellular atrophy, congestion and fatty metamorphosis. Some effects in the kidney, i.e., chronic inflammation, were also reported in the chronic NTP study (1982) in rats. NOAEL in rats ranged from 1 – 40 mg/kg bw/day and in mice from 2 – 40 mg/kg bw/day. The effective dose ranged between 5 and 100 mg/kg bw/day in rats and between 10 and 100 mg/kg bw/day in mice, being within the CLP Regulation guidance value range for classification in Category 2, after extrapolation to a 90-day exposure when required. In contrast, no severe adverse effects were reported in dogs in a 97-day study (Quast *et al*., 1983) at doses up to 25 mg/kg bw/day. Findings from the Quast *et al.* (1983) studies both in rats and dogs did not show effects warranting classification for STOT RE.
Regarding the inhalation route of exposure (Table 23 of the CLH report), results from the Klimisch 1 NTP (2015) whole-body inhalation studies in rats and mice are thoroughly discussed below and are considered as key evidence for classification by RAC, especially the long-term effects observed in the 13-week NTP (2015) study.

Tested concentrations in the two-week studies in rats and mice were 0, 100, 200, 400, 800, 1600 mg/m^3 and 0, 25, 50, 100, 200, 400 mg/m^3 in the 13-week (91-day) studies. Mortality and body weight gain in these studies are summarised in the following table. The findings from the high quality 90-day studies can be directly (i.e., without extrapolation of duration) compared to guidance values for STOT RE classification. In rats, no treatment-related effect in mortality and body weight gain was observed at doses where STOT RE effects were seen, indicating no general systemic toxicity. In mice, in the 13-week study, at 50 mg/m^3, no mortality was noticed in male or female mice, but reduction in body weight gain exceeded 15% for males and 20% for females compared to controls. During the study duration, though, dosed animals continued to gain weight. Therefore, any toxicity identified in target organs hereafter, could be regarded as the primary effect due to repeated exposure to VDC.

***Table**: Mortality and body weight gain at all doses in the 2-week and in the 13-week NTP (2015) study in rats and mice*

Dose (mg/m^3)	Rats				Mice			
	Males	Females	Males	Females	Males	Females	Males	Females
	Survival		Body weight gain (%)		Survival		Body weight gain (%)	
2-week NTP 2015 study								
0	5/5	5/5	69.9	47.6	5/5	5/5	15.2	12.7
100	4/5	5/5	64.8	48.8	5/5	5/5	3.4	9.5
200	5/5	5/5	72.8	45.2	4/5	5/5	5.5	10.3
400	5/5	5/5	65.6	41.0	**0/5**	4/5		13.3
800	**0/5**	**0/5**			**0/5**	**0/5**		
1.600	**0/5**	**0/5**			**0/5**	**0/5**		
13-weeks NTP 2015 study								
0	10/10	10/10	194	111	10/10	10/10	69.8	79.6
25	10/10	10/10	202	114	10/10	10/10	61.5	**57.9**
50	10/10	10/10	204	117	10/10	10/10	**53.0**	**58.7**
100	10/10	10/10	190	112	10/10	10/10	**43.2**	**56.1**
200	10/10	10/10	206	116	8/10	10/10	**44.1**	**46.4**
400	10/10	10/10	190	103		6/10		**53.3**

Numbers in bold represent high differences

Microscopic lesions of the nose were noted in both sexes of rats. Effects in the nose were reported in the 13-week study at all tested concentrations (some of the following effects from 25 mg/m^3), including olfactory epithelium atrophy, mineralisation, necrosis and turbinate atrophy. These

effects were of minimal severity at low doses, but the severity increased with increased concentrations for most of the effects observed. The nose was also the target organ in male and female mice in the 2-week study. Lesions in the nose included respiratory epithelial necrosis in all dosed males and in females at the highest doses. In addition, relative lung weights in the two-week study increased in males even at the lowest dose (15%), with a statistically significant ($P<0.01$) increase of 18% at the top dose, whereas in females a non-significant increase 7.7-11% at all doses was noted. In the 13-week study, relative lung weights remained practically unchanged in both sexes.

Effects in kidneys were also seen (renal tubules casts) at higher doses (800 mg/m^3). In rats, increased kidney weights were observed in both sexes. Clinical chemistry in the 13-week study supported the effects in kidneys. Exposure concentration-related minimal to mild increases (≤10%) were observed in total protein and globulin concentrations on days 3 and 23 in both male and female rats in various exposed groups. In addition, albumin and urea nitrogen was minimally increased during the first 25 days of the study. The total protein, albumin, globulin, and urea nitrogen concentrations returned to chamber control levels by week 14 and were consistent with possible mild dehydration, not confirmed as water consumption was not recorded.

In rats, adverse effects were described in the liver, characterised by centrilobular cytoplasmic alteration from mild to moderate severity in the two-week study from 100 mg/m^3, and from minimal to mild severity in the 13-week study from 50 mg/m^3.

In mice, effects were consistently observed in kidney, liver and nose. Critical effects were reported in the kidneys in males, with renal tubule necrosis, granular casts and renal tubule regeneration in the 2-week study from 100 mg/m^3. At this concentration no general systemic toxicity was observed. In the 13-week study, nephropathy was reported from 50 mg/m^3, which was characterised by minimal to mild tubule necrosis and cast formation, renal tubule regeneration, mild inflammatory infiltrates of lymphocytes, macrophages, and neutrophils within the interstitium and subcapsular areas, along with occasional tubule mineralisation. At this concentration, no mortality was noticed in male or female mice, but reduction in body weight gain exceeded 15% for males and 20% for females compared to controls. During the study duration, though, dosed animals continued to gain weight.

In the two NTP (2015) studies, relevant effects were also described in the nose, with necrosis of the respiratory epithelium (2-week study, in males at ≥ 200 mg/m^3) or respiratory epithelium squamous metaplasia (13-week study, in males and females at ≥ 100 mg/m^3). In all surviving groups of exposed females in the 2-week study, absolute and relative lung weights were significantly greater (up to 36%) compared to controls. In the 13-week study, gross lesions potentially related to exposure were observed in the lung of 5 female mice, including pale to white, 1 to 7 mm diameter foci. In the same study, the respiratory system as a whole appeared vulnerable, with laryngeal lesions (necrosis and respiratory epithelium hyperplasia and squamous metaplasia) being observed at early death in 400 mg/m^3 exposed females and bronchial epithelium necrosis and histiocytic inflammation of the lung in the same group.

Liver necrosis was noted in the 2-week study from 400 mg/m^3 for both sexes (minimal for surviving animals, marked for all early-death mice). In the 13-week study minimal necrosis for females from 50 mg/m^3 to marked necrosis at 400 mg/m3 ($p<0.01$) was reported, whereas for males, mild necrosis at 200 mg/m^3 was observed.

In the table below, the main target organs recognised for each relevant study described in the CLH report, are summarised, along with the classification attributed. Regardless of the Klimisch rating of the studies, even for those of lower quality (due to technical issues, such as use of only one dose, lack of experimental details etc.), the reported findings qualitatively support the results of the NTP studies, in particular by identifying the liver and kidney as the main target organs.

Details are also provided regarding the organs monitored in each study in order to assess more carefully potential effects in any other target organs than those already identified.

Table: *Summary of the main target organs following inhalation of VDC, the effective dose and the classification attributed for each relevant study described in the CLH report*

Inhalation Study	**Effective dose (respective effects)**	**Target organ toxicity relevant for classification***	**Organs monitored**	**Classification category based on the dose causing target organ toxicity relevant for classification***
** in case of multiple doses and/or multiple effects in parenthesis the minimum dose where the respective effect is observed, is mentioned*				
Rats				
NTP, 2015 Klimisch 1 2 weeks 6 h/day, 5 days/week	100 mg/m^3 (0.1 mg/L) (Centrilobular cytoplasmic alteration in the liver)	*Liver* Centrilobular cytoplasmic alteration (100 mg/m^3) *Kidney* Renal tubule, casts (800 mg/m^3)	Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. In addition to gross lesions and tissue masses, the eyes, kidney (except 50 ppm female mice), liver, lung, and nose were examined to a no-effect level.	**Category 1** (C ≤ 0.2 mg/L/6h/day) Liver: 0.017mg/L < 0.2 mg/L
NTP, 2015 Klimisch 1 13 weeks 6 h/day, 5 days/week	25 mg/m^3 (0.025 mg/L) (Olfactory epithelium mineralisation/ atrophy in the nose)	*Nose* (25 mg/m^3) Olfactory epithelium atrophy, mineralisation, necrosis and turbinate atrophy *Liver* Centrilobular cytoplasmic alteration/ vacuolisation (males 50 mg/m^3 L; females 200 mg/m^3)	The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Complete histopathologic examinations. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric,	**Category 1** (C ≤ 0.2 mg/L/6h/day) Nose: 0.025 mg/L < 0.2 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	
Rampy et al., 1977 /Quast et al, 1986 Klimisch 2 18 months 6 h/day, 5 days/week	300 mg/m^3 (0.3 mg/L)	*Liver* Hepatocellular fatty change consistent in males and females	At terminal necropsy, the brain, heart, liver, kidneys, and testes were weighed. Microscopic examinations were generally conducted on the followings organs: accessory sex glands, adipose tissue, adrenals, aorta, bone marrow (sternal), brain, epididymides, esophagus, heart, intestines (large and small), kidneys, liver, lungs, lymph nodes (mesenteric, mediastinal), mammy gland, nasal turbinates, ovaries, oviduct, pancreas, parathyroid, peripheral nerve, pituitary gland, prostate, salivary glands, skeletal muscle, skin, eye, spleen, spinal cord,	**No classification** (C >1.0 mg/L/6h/day) Liver: 1.8 mg/L>1.0 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			sternum, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, and any gross lesion or mass.	
Lee et al., 1977 Klimisch 3 <u>12 months</u> 6 h/d, 5 days per week	220 mg/m^3 (0.22 mg/L) (only one dose tested)	*Liver* A mild to markedly severe focal, disseminated vacuolisation, probably fatty change	Gross examination was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination.	**Category 2** (0.2 < C ≤ 1.0 mg/L/6h/day) Liver: 0.2 < 0.88 mg/L < 1.0 mg/L
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 <u>52 weeks</u> 4 h/day, 4-5 days/week	600-800 mg/m^3 (0.6-0.8mg/L)	*Liver* Hepatocyte vacuolisation, cloudy swelling, fatty degeneration, necrobiosis and necrosis	Complete autopsy Histological examinations on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Cytological	**No classification** (C >1.0 mg/L/6h/day) 1.6-2.1 mg/L>1.0 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			examinations were carried out on the bone marrow of the femur.	
Prendergast et al., 1967 Klimisch 3 13 weeks Continuous exposure	189 mg/m^3 (0.189 mg/L)	*Liver* (189 mg/m^3) Fatty metamorphosis, focal necrosis, haemosiderosis deposition, lymphocytic infiltration, bile duct proliferation and fibrosis *Kidney* (189 mg/m^3) Nuclear hypertrophy of the tubular epithelium	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) 0.758 mg/L<0.2 mg/L
Anonymous, 2004 13 weeks 6 h/day, 5 days/week	400 mg/m^3 (0.4 mg/L)	*Liver* Mononuclear cell aggregates and necrotic hepatocytes, hepatocellular vacuolation and hepatocellular hypertrophy	Organ weight (liver), gross examination at necropsy, microscopic examination of liver and muscle, and detailed microscopic examination of peripheral nervous system organs were evaluated.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0,2 < 0.4 mg/L < 1.0 mg/L
Anonymous, 1979h Klimisch 4 30 days	-	-	Macroscopic observation, organ weight (heart, liver, kidney, spleen, testis, thyroid, adrenal gland and lung), body weight (on 20 rats) and histology on major organs (heart, aorta, trachea, lung, esophagus, stomach, small intestine, colon, parotid glandular, liver, pancreas, spleen, thymus, lymph nodes, epididymis prostate, semen bubble, ovaries, uterus, skeletal muscle, teeth, skin, eye with	**No classification**

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			optic nerve) were conducted	
Gage et al., 1970 Klimisch 4 <u>4 weeks</u> 6h/day, 5 days/week	2000 mg/m^3 (2 mg/L)	*Liver* (2000 mg/m^3) Cell degeneration *Nose (starting at 800 mg/m^3)* Slight nose irritation with no significant findings noted at the autopsy, *not relevant for classification alone*		**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.62 mg/L<1.0 mg/L
Mice				
NTP, 2015 Klimisch 1 <u>2 weeks</u> 6 h/day, 5 days/week	100 mg/m^3 (0.1 mg/L) (Kidney effects)	*Liver* (males: 200 mg/m^3, females 400 mg/m^3) Hepatic necrosis *Kidney* (100 mg/m^3) Minimal to moderate renal tubule necrosis and granular casts Mild to moderate renal tubule regeneration *Nose* (from 200 mg/m^3 in males only) Minimal necrosis of the respiratory epithelium	Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. Histopathology In addition to gross lesions and tissue masses, the eyes, kidney (except 50 ppm female mice), liver, lung, and nose were examined to a no-effect level.	**Category 1** (C ≤ 0.2 mg/L/6h/day) Kidney: 0.017 mg/L < 0.2 mg/L
NTP, 2015 Klimisch 1 <u>13 weeks</u> 6 h/day, 5 days/week	50 mg/m^3 (0.05 mg/L) (Minimal to moderate nephropathy in males)	*Liver* Necrosis (50 mg/m^3) (from individual hypereosinophilic hepatocytes with nuclear pyknosis and karyolysis to hypereosinophilic coagulum) Centrilobular hepatocyte hypertrophy (female 400 mg/m^3) *Kidney* Renal tubule necrosis, protein cast formation in mice that experienced early death and nephropathy in those surviving to terminal kill. Marked renal tubules necrosis (200 mg/m^3)	The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Complete histopathologic examinations In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum,	**Category 1** (C ≤ 0.2 mg/litre/6h/day) Kidney: 0.05 mg/L<0.2 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
		Minimal to moderate nephropathy in males (50 mg/m^3) *Nose (only females* 400 mg/m^3*)* Minimal to moderate necrosis of the nasal respiratory epithelium and minimal turbinate atrophy *Lung (only females 400 mg/m^3)* Bronchial epithelium necrosis Histiocytic inflammation *Larynx* Necrosis and respiratory epithelium hyperplasia (400 mg/m^3) and squamous metaplasia (100 mg/m^3)	jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	
Lee et al., 1977 Klimisch 3 <u>12 months</u> 6 h/day, 5 days/week	220 mg/m^3 (0.22 mg/L) (only one dose tested)	*Liver* Enlarged and basophilic hepatocytes with enlarged nuclei, large round eosinophilic inclusions; mitotic figures or polyploidy; microfoci of mononuclear cells; focal degeneration and necrosis	Gross examination, especially for any appearance of abnormal growth or other lesions, was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed,	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.2 < 0.88 mg/L < 1.0 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			sectioned, and stained for microscopic examination.	
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 52 weeks	Effects observed from 40 mg/m^3 but even in controls, no statistical difference	*Liver (*considered not treatment related and not relevant for classification*)* Hepatocyte vacuolisation, cloudy swelling, fatty degeneration, necrobiosis and necrosis) and amyloidosis *Kidney (*considered not treatment related and not relevant for classification*)* Cloudy swelling and necrosis), amyloidosis of glomeruli and chronic nephritis	A complete autopsy is carried out on each animal. Histological examinations are performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations are carried out on the bone marrow of the femur.	**No classification**
Hamster				
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 52 weeks	NOAEC=100 mg/m^3	No effects observed	A complete autopsy was carried out on each animal. Histological examinations were performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological	**No classification**

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			examinations were carried out on the bone marrow of the femur.	
Prendergast et al., 1967 Klimisch 3 <u>13 weeks</u> Continuous exposure	Guinea Pigs 189 mg/m^3 (0.189 mg/L)	*Liver (*effects of low toxicological significance*)* ↑SGPT ↑alkaline phosphatase	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**No classification**
	Monkeys 189 mg/m^3 (0.189 mg/L)	*Liver* Fatty metamorphosis, focal necrosis, haemosiderosis deposition, lympholytic infiltration, bile duct proliferation, fibrosis	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney as well as on sections of brain, spinal cord, and adrenal gland were taken for histopathologic evaluation.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.758 mg/L<0.2 mg/L
	Rabbits NOAEC = 189 mg/m^3	No effects observed	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**No classification**
	Dogs 189 mg/m^3 (0.189 mg/L)	*Liver* Fatty metamorphosis, focal necrosis, haemosiderosis deposition, lympholytic infiltration, bile duct proliferation, fibrosis	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney as well	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.758 mg/L<0.2 mg/L

Inhalation Study	Effective dose (respective effects)	Target organ toxicity relevant for classification*	Organs monitored	Classification category based on the dose causing target organ toxicity relevant for classification*
			as on sections of brain, spinal cord, thyroid gland and adrenal gland were taken for histopathologic evaluation.	

**Comparison of the extrapolated effective dose to 90-day/6-hr exposure with the Guidance Value for each category*

In conclusion, as no human data is available, reliable data on experimental animals after repeated exposure to VDC will be used for classification purposes. As described above, inhalation appears to be the most detrimental route of exposure for VDC. The available oral studies support the classification in Category 2 at best, whereas several studies by inhalation, and particularly the most reliable ones (NTP, 2015) support classification in Category 1. The classification category of VDC should therefore be based on the studies performed via the inhalation route, according to the CLP guidance (2017).

The four studies in rats and mice performed by the NTP in 2015 (two-week and 13-week) are studies of high quality, that are used as the key studies to conclude on the classification of VDC.

The nose effects from 0.025 mg/L in rats described in the NTP (2015) 13-week study (olfactory epithelium necrosis, olfactory epithelium and turbinate atrophy) can be regarded as of significant toxicity and, along with the cluster of effects in lungs and trachea observed, do thus lead to a Category 1 classification. In the same way, renal tubule necrosis, granular casts and renal tubule regeneration (from 0.1 mg/L in the 2-week) and nephropathy (from 0.05 mg/L at the 13-week study) based on a cluster of effects observed in mice, as explained above, are also considered significant effects, which lead to classification in Category 1. Moreover, in the 2-week rat study, the critical adverse effects leading to classification were observed in the liver, occurred from 0.1 mg/L and were described as centrilobular cytoplasmic alteration from minimal to mild severity. At higher concentrations the severity increased and centrilobular necrosis was observed consistent with a more severe stage of hepatocellular damage. Liver alterations are therefore considered as significant effects clearly indicating toxicologically relevant functional disturbance of the organ. The results of the other studies of lower Klimisch rating described in the table above, confirm the target organs of VDC toxicity being the liver, kidney and respiratory tract.

The LOAEC Associated with some of these critical effects are 0.025 mg/L in the 13-week rat study and 0.05 mg/L in the 13-week mouse study. Due to the CLP Regulation guidance values for classification in category 1 via the inhalation route (C ≤ 0.2 mg/L), these 13-week studies lead to a classification of VDC in Category 1. The LOAEC for some of the critical effects in rats and mice in the 2-week studies (NTP, 2015) is 0.1 mg/L and lead consistently to the same category of classification.

Therefore, RAC considers that classification of VDC as **STOT RE 1; H372: Causes damage to organs through prolonged or repeated exposure (respiratory tract, kidney, liver)** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The data set for mutagenicity was quite extensive and it was evaluated thoroughly by the DS.

In vivo, there was a recent well-performed Comet assay of high reliability, conducted according to OECD TG 489. It demonstrated that VDC induced DNA damage in the lungs, liver and kidneys of male rats, after inhalation.

In vitro, VDC was found to be mutagenic in different strains (S. thyphimurium TA98, TA100, TA1535, TA1530, BA13, BAL13, TA1537, TA1538, TA92, E. coli K-12, E. coli WP2, depending on the study considered) generally in the presence of an exogenous metabolising system (metabolic activation), either from liver, kidney or lung of mice or rats. VDC was also shown to be mutagenic in yeast models in the presence of induced mouse liver S9, increasing the rate of gene mutations and gene conversions. In mammalian cells, VDC was shown to be mutagenic in the MLA tk+/- test on L5178Y cells with S9 from induced rat liver. These results supported the positive findings observed in the *in vivo* Comet assay, especially since the liver, the kidney and the lung express several enzymes involved in the metabolism of VDC to mutagenic metabolites, such as epoxides, as explained in the toxicokinetics section.

In conclusion, VDC was found to be mutagenic in an *in vivo* somatic cell genotoxicity test and in various *in vitro* mutagenicity assays. Therefore, the criteria for a classification in Category 2 were considered fulfilled. Consequently, the DS proposed classification as Muta. 2; H341: Suspected of causing genetic defects.

Comments received during consultation

Two comments were received.

One MSCA supported the proposed classification and reasoning by the DS.

One comment was received from an Industry/Trade Association disagreeing with the proposed classification. While the commenting Industry/Trade Association acknowledged the positive *in vitro* results as well as the positive *in vivo* Comet assay for VDC, the following arguments were presented:

- Four reliable (Klimisch 2 and 3) studies (*in vivo* micronucleus tests, chromosomal aberration test) were negative: the *in vivo* micronucleus tests on bone marrow or circulating erythrocytes conducted in mice and the chromosomal aberration test on rat bone marrow cells did not show any evidence of chromosomal aberrations.
- Even though VDC was shown to reach the bone marrow in one of the above-mentioned micronucleus tests, it did not induce any genotoxic effects.
- Two dominant lethal (DL) assays showed negative results.
- A Sex-Linked Recessive Lethal Mutation *in vitro* assay on Drosophilia melanogaster was negative.

Hence, the Industry/Trade Association concluded that VDC was not expected to induce heritable genetic damage (chromosomal aberrations or gene mutations) and considered that classification was not warranted in accordance with the CLP Regulation.

The DS replied that according to the CLP Regulation where there is evidence of only somatic cell genotoxicity, substances should be classified as suspected germ cell mutagens (i.e., Category 2). Classification as a suspected germ cell mutagen may also have implications for potential carcinogenicity classification. This was considered to hold true especially for those genotoxicants which were incapable of causing heritable mutations because they could not reach the germ cells

(e.g., genotoxicants only acting locally, 'site of contact' genotoxicants). This was considered to be the case for VDC where the genotoxic effects *in vivo* were seen locally only in detoxification organs. The DS clarified that if positive results *in vitro* were supported by at least one positive local in vivo somatic cell test, such effects should be considered as sufficient justification to classification in Category 2.

Moreover, the DS added that the negative *in* vivo studies did not investigate the same endpoints and the same organs as the comet assay, and this could according to the DS explain the results observed. A comet assay is the only assay allowing the identification of site of contact genotoxicants.

Assessment and comparison with the classification criteria

All the available *in vitro* and *in vivo* studies presented in Tables 14, 15 and 16 (pages 23-29) of the CLH report were evaluated by RAC.

In vitro

In bacterial test systems, VDC consistently demonstrated mutagenic activity when tested in the presence of a metabolic activation system, in a closed environment to control volatility. Many of these studies were old, had methodological limitations and were not reported in adequate detail, but still allowed to assess a genotoxic profile of VDC.

VDC was shown to be mutagenic in different strains (S. thyphimurium TA98, TA100, TA1535, TA1530, BA13, BAL13, TA1537, TA1538, TA92, E. coli K-12, E. coli WP2), generally in the presence of an exogenous metabolising system either from liver, kidney or lung from mice or rats.

Mutagenicity was higher in the presence of liver mouse S9, but lower when mouse kidney or lung S9 fractions were used (Bartsch *et al*., 1975). Phenobarbital, a potent cytochrome P450 inducer, in accordance with the analysis in the toxicokinetics section, increased mutagenic responses in tests using mouse liver, kidney, and lung S9 (Bartsch *et al*., 1975). Similarly, VDC was mutagenic in strains TA100 and TA1535 (Baden *et al*., 1978, 1982), in which pre-treatment with CYP inducers increased the effectiveness of mouse liver and kidney S9, with mouse liver S9 also being more effective than rat liver S9.

Positive results were also observed in S. typhimurium strains TA92, TA98, TA100, TA1535, and TA1537 and E. coli strain WP2 uvrA in the presence of human S9 or Swiss mouse liver S9 (Oesch *et al*., 1983).

In yeast models, S. cerevisiae D7, VDC was mutagenic only in the presence of induced mouse liver S9, increasing the rate of gene mutations and gene conversions (Koch *et al*., 1988; Bronzetti *et al*., 1981). VDC also induced aneuploidy in S. cerevisiae D7 in the absence of S9 as visualised by chromosome malsegregation (Koch *et al*., 1988).

On the contrary, VDC (tested at up to 6666 µg/plate) was not mutagenic in strains TA98, TA100, TA1535, or TA1537, with or without induced S9, when a preincubation protocol was used (Mortelmans *et al*., 1986). However, there are uncertainties on whether the conditions used in this specific study could completely account for the high volatility of VDC.

In mammalian systems inconsistent mutagenic responses were seen in L5178Y mouse lymphoma cells with VDC in the absence of metabolic activation; with activation, both cytotoxicity and mutagenicity were consistently positive in several experiments.

VDC did not induce gene mutations in V79 cells with or without metabolic activation from rat and mice liver. VDC stimulated unscheduled DNA synthesis in isolated rat hepatocytes in primary culture. VDC also induced single-strand breaks in phage PM2 DNA after 62-h incubation.

Strong, dose-related increases in chromosomal aberrations were seen in cultured Chinese hamster lung (CHL) cells exposed to VDC in the presence of induced rat liver S9. In addition, VDC induced relatively weak sister chromatid exchanges (1.6/1.8-fold), but significant increase in CHL cells only in the presence of induced rat liver S9. It is noted that in the chromosomal aberration study, metyrapone (an inhibitor of the P-450 activity) was added to the culture at various concentrations together with S9 mix. The authors reported that the frequencies of aberrant cells decreased as the concentration of metyrapone increased (0.1-1.0 mM), thus, confirming that cytochrome P-450 in the liver microsome participates in the activation of VDC.

In vivo

The mutagenicity/genotoxicity of VDC has been investigated in several *in vivo* studies. The most recent and reliable study is a comet assay performed according to OECD TG 489 in male Wistar Han rats exposed by nose-only inhalation to VDC at 25, 250, 750 and 6350 ppm (corresponding to 0.1, 1, 3 and 25 mg/L) for 4 hours per day for 3 days (Anonymous 2016, Klimisch 1). Lung, liver, kidney and bone marrow cells were recovered between 2 and 6 hours after the last exposure. In the following table, data on viability of single cell suspensions is provided:

Table*: Sigle cell suspensions viability in the comet assay in Anonymous (2016)*

Animal #	**Dose**	**Viability (%)**			
		Kidney	*Liver*	*Lung*	*Bone Marrow*
1	0	98	100	100	98
6	25 mg/L	99	99	100	96
11	200 mg/kg (EMS)	96	100	100	99
16	0	95	100	100	95
21	25 mg/L	94	100	99	97
26	200 mg/kg (EMS)	99	98	100	92
31	0	100	100	100	96
36	25 mg/L	97	98	97	98
41	200 mg/kg (EMS)	100	100	98	98
46	0	100	100	100	100
51	25 mg/L	100	100	100	100
56	200 mg/kg (EMS)	100	100	100	84

In this assay, VDC induced a statistically significant increase in DNA damage in lung, liver and kidney (at all concentrations in the kidney and the lung and from 1 mg/L in the liver). Results are summarised in the following table.

***Table**: Histopathology and DNA damage results in the comet assay in Anonymous (2016)*

Organ	Dose (mg/L)	Histopathology Findings		Comet Assay (DNA Damage)			
		Severity	Description	Relevance	Tail Intensity (SD)		
					Treated Group	Negative Control	Positive Control
Lung	25	Severe	Degeneration/regeneration bronchiolar epithelium Lymphoid depletion BALT Inflammatory cell infiltrate peribronchial	Relevant	32.0 (24.4)*	4.61 (0.69)	87.0 (12.2)***
	3	Minimal	Regeneration bronchiolar epithelium Inflammatory cell infiltrate peribronchial	Relevant	50.0 (7.39)***	19.7 (9.75)	93.4 (1.80)***
	1	-	-	Relevant	81.6 (3.31)***	15.0 (7.08)	90.6 (2.81)***
	0.1	-	-	Not relevant	5.65 (1.38)	4.73 (1.01)	82.6 (3.43)***
Liver	25	Severe	Single cell necrosis, centrilobular/ bridging	Relevant	20.0 (7.94)***	3.65 (3.34)	92.7 (4.80)***
	3	Severe	Single cell necrosis, centrilobular/ bridging Hepatocellular hypertrophy, centrilobular	Relevant	33.3 (8.93)***	15.4 (4.30)	99.0 (0.21)***
	1	Severe	Single cell necrosis, centrilobular/ bridging Hepatocellular hypertrophy, centrilobular	Relevant	44.4 (16.9)*	16.4 (14.7)	94.5 (1.62)***
	0.1	Not adverse	Hepatocellular hypertrophy, centrilobular Cytoplasmic alteration centrilobular	Not relevant	16.9 (4.08)	14.4 (5.50)	86.8 (2.86)***
Kidney	25	Severe	Tubular degeneration	Relevant	68.7 (13.8)***	4.37 (0.76)	97.0 (1.55)***
	3	-	-	Relevant	57.2 (2.56)***	11.6 (2.56)	96.6 (1.89)***
	1	-	-	Relevant	35.6 (7.90)*	26.6 (5.07)	92.9 (1.09)***
	0.1	-	-	Relevant	55.0 (11.0)***	13.8 (3.52)	87.2 (3.24)***

Bone Marrow	25	-	-	Not Relevant	4.00 (0.95)	4.32 (2.08)	80.3 (10.0)***
	3	-	-	Questionable	11.6 (3.18)**	5.31 (1.30)	85.4 (2.81)***
	1	-	-	Not Relevant	11.9 (4.42)	10.2 (1.99)	82.2 (2.56)***
	0.1	-	-	Not Relevant	12.8 (7.10)	10.0 (3.02)	78.2 (3.16)***

** p<0.05; ** p<0.01; *** p<0.001 (Student t test)*

Histopathological lesions (from minimal to severe in severity) were also observed in these organs (at the highest dose in kidney, the two highest doses in lung, and the three highest doses in liver), but, in accordance with the OECD TG, this does not confound the relevance of the DNA damages observed, taking also into account the fact that the observed DNA damages occurred also at concentrations below to those inducing histopathological findings. A trend test was not performed by the authors to assess if the DNA damages were concentration related. It is noted, that for each tested concentration different negative controls were used. DNA damages observed in liver, kidney and lung are consistent with the fact that VDC is extensively metabolised into genotoxic metabolites in these tissues. In contrast, no DNA damage was seen in bone marrow. However, under these experimental conditions bone marrow exposure to VDC was not confirmed by the study authors.

In the Sawada *et al*. (1987) micronucleus study, there was a 23% decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (PCE/NCE ratio) at 200 mg/kg indicating that under the conditions of the specific study (i.e., gavage administration) the bone marrow of male mice was probably exposed to VDC. It is noted that in the OECD TG 474 Mammalian Erythrocyte Micronucleus Test, "the highest dose may also be defined as a dose that produces toxicity in the bone marrow (e.g., a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood of more than 50%, but to not less than 20% of the control value)".

In other *in vivo* studies no evidence of genotoxicity was seen after VDC exposure. The various micronucleus tests on bone marrow cells or circulating mouse erythrocytes were negative, as well as the chromosomal aberration test on rat bone marrow cells (i.p. administration in Sawada *et al*., 1987; whole-body inhalation exposure in NTP, 2015 and in Quast *et al*., 1986).

In these studies, there was no evidence that the target organ, i.e., the bone marrow, was adequately exposed (lack of/slight toxicity, no change in the percentage of PCE/NCE). Therefore, as previously discussed for the comet assay, negative results on bone marrow cells cannot be used to demonstrate the absence of genotoxicity. In addition, most of the studies did not report the inclusion of a positive control to validate the results.

Negative results were also reported in dominant lethal tests (assays for mutagenicity in germ cells) in male CD-1 mice (Anderson *et al*., 1977) and in male Crl:CD(SD) rats exposed to VDC by inhalation (Short *et al*., 1977).

VDC did not induce increases in sex-linked recessive lethal mutations in germ cells of adult male Drosophila melanogaster exposed via feed or injection (Foureman *et al*., 1994). However, the study had several reporting deficiencies.

Given the extensive hepatic metabolism of VDC to potentially genotoxic metabolites as suggested by the results of the *in vitro* tests, it is unlikely that these highly reactive metabolites would be able to reach the bone marrow or the germ cells via the blood stream, thus explaining most of the negative results presented above.

Although in the available toxicokinetic studies there is no evidence that the substance or its reactive metabolites reach and interact with the genetic material of germ cells, in the subchronic inhalation NTP study (2015), it was shown that VDC had effects on the male reproductive system both in rats and mice. More specifically, spermatid heads (106/g testis) decreased 14.6% at 100 ppm, while at 25 ppm a decrease of 9.18% is recorded. Similarly, sperm motility was decreased by 2.5-4.5%. However, RAC is of the opinion that these results do not constitute robust evidence of the ability of VDC to interact with germ cells.

In conclusion, the table below summarises the *in vitro* and in *vivo* studies rendering positive results.

Table: *Summary of positive results in the mutagenicity battery of tests*

Test Organism	Test System		Endpoint
In vitro			
Bacteria	TA1535, TA1537, TA98, TA100 and TA92	+ S9	Reverse gene mutation
Bacteria	E. coli WP2	+ S9	Reverse gene mutation
Bacteria	E. coli K-12	+ S9	Reverse gene mutation
Bacteria	BA13 (mutation indicator) and BAL13 (survival indicator)	+ S9	Forward gene mutation
Yeast	S. cerevisae strain D7	+ S9	Reverse gene mutation
Yeast	S. cerevisae strain D7	+ S9	Gene Conversion
Yeast	D61.M	+/- S9	Chromosomal aberration (Aneuploidy)
Mammalian	L5178Y cells	+ S9	Forward gene mutation
Mammalian	Rat hepatocytes	- S9	DNA damage/repair (UDS)
Mammalian	Chinese hamster, lung	+ S9	Chromosomal damage (CA test)
Mammalian	Chinese hamster, lung	+ S9	Chromosomal damage (SCE assay)
Bacteria	Double-stranded circular phage PM2 DNA molecule	+ S9	DNA damage/repair (single-strand break)
In vivo			
Male Wistar Han rats	Comet assay in lung, liver, kidney and bone marrow cells	-	DNA damage

Several of the available *in vitro* studies were positive in the presence of exogenous metabolic activation and provide evidence for the mutagenic properties of VDC. VDC induced gene mutations in bacteria systems, yeast models and in mouse lymphoma cells, it was positive in a UDS test in rat hepatocytes, induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster lung cells and induced aneuploidy in the presence and absence of metabolic activation in a single study in Saccharomyces cerevisiae.

In contrast, in the available *in vivo* assays, gene mutation was detected only in the comet assay, supporting the concern raised by the results of the *in vitro* studies. This assay was positive in lung (site of contact), liver and kidney. These findings are consistent with the proposed metabolic pathway of VDC generating mutagenic metabolites (such as epoxides) and the excretion pattern

followed. In addition, the positive *in vitro* mutagenic results obtained only in the presence of metabolic activation support the mutagenicity of VDC metabolites.

Comet assay is a test which identifies chemicals that induce primary DNA damage. Under alkaline conditions (> pH 13), the comet assay can detect single and double strand breaks resulting, for example, from direct interactions with DNA alkali labile sites, as a consequence of transient DNA strand discontinuities resulting from DNA excision repair, or from processing during the assay. These DNA strand breaks may be: 1) repaired, resulting in no persistent effect; 2) lethal to the cell; or 3) fixed as a mutation resulting in a permanent heritable change. Therefore, the alkaline comet assay detects primary DNA strand breaks that do not always lead to gene mutations and/or chromosomal aberrations and cannot provide conclusive evidence for classification in Category 1B on its own.

In the *in vivo* dataset of VDC, there are no other positive results in either somatic or germ cells (micronucleus tests, chromosomal aberrations tests or dominant/sex-linked recessive lethal mutation tests). VDC did not induce genotoxic effects in bone marrow cells or male germ cells. However, as already mentioned it is not certain that the substance reached these target tissues.

Regarding classification of VDC for mutagenicity, classification in Category 1A is not warranted based on the absence of human data.

A classification in Category 1B is not justified because no *in vivo* heritable mutagenicity studies in mammals or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells, are available.

VDC was found to fulfil the CLP criteria for a classification in Category 2 based on:

- in an *in vivo* somatic cell (lungs, liver and kidneys) genotoxicity test (comet assay performed according to OECD TG 489)
- in various *in vitro* mutagenicity tests (mainly Ames tests) mostly in the presence of metabolic activation

In conclusion, RAC considers that **classification as Muta. 2; H341: Suspected of causing genetic defects is warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Even though there are several studies in the literature assessing the carcinogenic properties of VDC, the DS considered the carcinogenicity studies performed by the NTP (1982, 2015) as the most reliable and relevant studies and thus as the as key studies for concluding on classification. The NTP assessed the statistical and biological significance of each tumour type observed after VDC exposure, as well as the link between the different neoplasms and the exposure to VDC to conclude on the overall strength of evidence for carcinogenicity. NTP assessed carcinogenic potency of VDC in rats and mice by oral route (1982) and by inhalation route (2015).

In the oral studies the only observed significant increase in tumour incidence occurred in low-dose female mice: lymphoma and lymphoma or leukaemia combined (NTP, 1982). These incidences were considered not to be related to VDC administration because similar effects were not found in the high-dose female mice or in male mice or rats. On the other hand, several significant non-neoplastic lesions were observed. The incidence of chronic inflammation in the kidney in both male and female rats was higher in high-dose animals than in controls. Although

this lesion is common in aging rats, the occurrence appeared to be dose related. In mice, necrosis of the liver was observed more frequently in dosed mice than in controls. Overall, NTP concluded that VDC administered by gavage was not carcinogenic. However, both NTP (1982) and IARC (2019) stated that this study should not be taken as a definitive proof that VDC is not a carcinogen because of the conditions used in the specific study.

As supporting evidence, the DS referred to Quast et al. (1983) who studied rats exposed to VDC in drinking-water for 2 years. No statistically significant increase in tumour incidence was reported, but the achievement of the maximum tolerated dose in order to be able to investigate carcinogenic effects was questionable according to the authors of the study. In addition, there were other available studies, however of limited quality, that did not report any increase in tumour incidence.

The DS shared the concerns raised by the NTP and IARC and considered that, even though the available studies by oral route did not report an increase in tumours, the high incidence and the severity of non-neoplastic lesions observed along with the low quality and almost 40-year-old studies available, carcinogenicity of VDC by oral exposure could not be excluded.

When carcinogenicity via the inhalation route was assessed in rats and mice in the NTP (2015) study, there was clear evidence of the carcinogenic activity of VDC in both species and sexes. In male rats increased incidences of malignant mesotheliomas were observed. Renal tubule carcinoma and respiratory epithelium adenoma in the nose of male rats were also considered to be related to VDC exposure. In female rats increased incidences of C-cell adenoma, carcinoma in the thyroid gland and systemic mononuclear cell leukaemia were reported, along with malignant mesothelioma. In male mice increased incidences of renal tubule adenoma and carcinoma and hepatocholangiocarcinoma were considered to be related to VDC exposure. Similarly, in female mice increased incidences of systemic haemangioma or haemangiosarcoma (combined) were reported. Overall, the DS considered all the above-mentioned tumorigenic activity relevant for classification purposes, except leukaemia reported in female rats.

The DS also evaluated other studies of low reliability and relevance, as supporting evidence. Maltoni *et al*. (1984) concluded that adenocarcinoma of kidney in mice was the only specific tumour linked to VDC exposure (rats, mice and hamsters were tested), being consistent with the results of the NTP studies in male rats and mice. This result was considered to confirm that kidney was a target organ of carcinogenesis of VDC. Additionally, the DS could not interpret the relevance of the reported increased incidence of mammary tumours in Quast *et al*. (1986) and Cotti *et al*., (1988) and tumours reported by Lee *et al*. (1977, 1978) and Hong *et al*. (1981) due to limited reliability of the studies.

Finally, regarding dermal exposure, the DS discussed the Van Duuren *et al*. (1979) study which investigated the carcinogenic potential of VDC by subcutaneous injection or dermal application in mice. No skin papillomas or local sarcomas were observed in the controls or treated mice. These experiments suffered from several limitations (Klimisch 3). The same team also tested VDC for its initiating activity in a two-stage mouse-skin assay (Van Duuren *et al*., 1979). Regarding these results, VDC could be seen as an initiating agent.

Based on all the above, and along with the fact that VDC is metabolised into mutagenic compounds (such as epoxides) and was proposed to be classified as a substance suspected of causing genetic defects (Muta. 2), the DS believed that the level of concern for human carcinogenicity following the strength of evidence analysis was high, and therefore, classification as Carc. 1B was considered fully justified.

Comments received during consultation

One comment by an MSCA was received during the consultation supporting the proposed classification as Carc. 1B; H350.

Assessment and comparison with the classification criteria

Human evidence

The DS did not discuss any human evidence in the CLH report. Nevertheless, in the IARC assessment of VDC, a good-quality cohort study was mentioned investigating health effects among 4806 workers in a plastics manufacturing plant in the USA, who were exposed to vinyl chloride, polyvinyl chloride (PVC) dust, VDC, and several other chemicals (Waxweiler *et al*. 1981). A significant increase in mortality due to lung cancer was observed but no association between lung cancer and exposure to VDC was reported. Two smaller occupational cohort studies (Ott *et al*., 1976; Thiess *et al*., 1979) with co-exposure to vinyl chloride monomer and acrylonitrile had significant limitations and were considered of limited value.

Animal studies

RAC considered all studies summarised by the DS in Tables 17-20 of the CLH report for the evaluation of the carcinogenic activity of VDC. Thirteen studies were performed in rats (8 via inhalation route, 5 via oral route), 7 in mice (5 via inhalation route, 1 via oral route, 1 via dermal route) and 2 inhalation studies on hamsters. Inhalation (whole body exposure) studies by NTP 2015 (Klimisch score 1) in rats and mice and those by Rampy *et al.* (1977), Quast *et al.* (1986), along with the oral (by gavage) NTP 1982 study and the oral study by Quast *et al.* (1983) study (in drinking water at libidum, Klimisch score 2) are considered of high reliability by RAC. The other studies were evaluated by the DS as Klimisch score 3. The carcinogenicity study via dermal exposure by Van Duuren *et al.* (1979) examined mice exposed to VDC cutaneously and sub-cutaneously. They also performed an initiation/promotion study using tetradecanoylphorbol-13-acetate as a pre-treatment agent, but the study was considered by DS as Klimisch score 3, mainly due to limited reporting details. In the following table a summary of the observed incidence of neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for VDC classification purposes as a carcinogen, is presented, as retrieved from all the studies evaluated in the CLH report.

Table: *Summary of the reported neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for the assessment of the carcinogenic properties of VDC*

Study	Target organ	Lowest dose tumours/lesions are observed	Neoplastic and non-neoplastic incidences	Mortality and body weight changes compared to controls
Oral route				
NTP, 1982 (Klimisch 2) Rats	Liver Kidney Pituitary Adrenals Pancreas Haematopoietic system	1 mg/kg bw/d	Primary tumours (most of them statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test, NOT significant when life table analyses used) *Males* Subcutaneous tissue – fibromas Haematopoietic system – leukaemia Pituitary – adenomas	Survival rates and body weight gains similar to controls In male rats 12 controls and 10 low-dose animals were accidentally killed during week 82 of the study NOAEL 5 mg/kg bw/day, the highest

Study	Target organ	Lowest dose tumours/lesions are observed	Neoplastic and non-neoplastic incidences	Mortality and body weight changes compared to controls
			Adrenal – Pheochromocytomas Pancreatic islets – Islet-cell adenomas and carcinomas Testis – Interstitial-cell Tumours *Females* Haematopoietic system – leukaemia, lymphomas Liver - Neoplastic nodule Pituitary – adenomas Adrenal – Pheochromocytomas <u>Non-neoplastic lesions</u> Chronic renal inflammation in both sexes (possible age-related)	exposure tested for carcinogenicity
NTP, 1982 (Klimisch 2) Mice	Haematopoietic system Liver	2 mg/kg bw/d	*Female* Haematopoietic system – leukaemia, lymphomas, malignant lymphomas <u>Non-neoplastic findings</u> Necrosis of the liver (focal, multifocal or diffuse) in high-dosed males and low-dosed females	Survival rates similar to controls Body weight gain in males similar to controls, in females 9.7% decrease at 2 mg/kg bw/day, 4.1% decrease at 10 mg/kg bw/day NOAEL 10 mg/kg-day, the highest exposure tested for carcinogenicity
Quast *et al*., 1983 (Klimisch 2) Rats	Liver	Males 20 mg/kg bw/d Females 9 mg/kg bw/d	Only non-neoplastic lesions in the liver Midzonal fatty change in females and males only at the 20 mg/kg bw/day	The mortality among the test animals was comparable to the controls Mean body weights of the rats over the 2-year period were similar for all groups
Ponomarkov & Tomatis, 1980 (Klimisch 3) Rats	Rare scattered incidences stomach liver rectal salivary gland	Pregnant females: 150 mg/kg bw once Males/Females Progeny: 50 mg/kg bw 1/week	*Pregnant females* Hyperplastic liver nodules (2/23) *Male progeny* 1 squamous-cell carcinoma of the stomach	Survival rates and body weight similar to controls

Study	Target organ	Lowest dose tumours/lesions are observed	Neoplastic and non-neoplastic incidences	Mortality and body weight changes compared to controls
			1 liver carcinoma 1 seminoma 1 rectal adenomatous polyp. Hyperplastic liver nodules 2/81 Meningiomas increased non-statistically significantly *Female progeny*, 2 liver cell carcinomas, and 1 liver cell adenoma 1 carcinoma and 1 adenoma of the salivary gland Hyperplastic liver nodules 6/80	
Inhalation route				
NTP, 2015 (Klimisch 1) Rats	Nose Kidney, Thyroid gland, Internal organs (mainly epididymis and testes), Liver	100 mg/m³	*Males* Malignant mesothelioma (mainly from the tunica vaginalis) Adenoma of the nasal respiratory epithelium Renal tubule carcinomas *Females* Malignant mesotheliomas (1 at 100 mg/m³, 1 at 200 mg/m³) Adenoma of the nasal respiratory epithelium. C-cell adenomas/carcinomas of the thyroid gland Mononuclear cell leukaemia	<u>Survival</u> • 100 mg/m³ *Males* 28/50 vs control 26/50 *Females* 27/50 vs control 34/50 • 400 mg/m³ *Males* 21/50 vs control 26/50 *Females* 21/50 vs control 34/50 <u>Body weight gain</u> • 100 mg/m³ *Males* Difference vs control – 0.2% *Females* Difference vs control + 4.5% • 400 mg/m³ *Males* Difference vs control – 3.8% *Females* Difference vs control + 0.8%
NTP, 2015 (Klimisch 1) Mice	Pulmonary system Nose Kidney Liver, Vascular system Small intestine Uterus	25 mg/m³	*Males*: Renal tubule adenoma, renal tubule carcinoma, renal tubule hyperplasia hepato-cholangiocarcinoma *Females*: Haemangioma and haemangiosarcoma of the vascular system Liver haemangiosarcoma	<u>Survival</u> • 25 mg/m³ *Males* 40/50 vs control 30/50 *Females* 26/50 vs control 37/50 • 100 mg/m³ *Males* 19/50 vs control 30/50 *Females* 26/50 vs control 37/50 <u>Body weight gain</u> • 25 mg/m³

Study	Target organ	Lowest dose tumours/lesions are observed	Neoplastic and non-neoplastic incidences	Mortality and body weight changes compared to controls
			Hepatocellular adenoma, carcinoma Hepato-cholangiocarcinoma Bronchioloalveolar carcinoma Carcinoma of the small intestine (ileum)	*Males* Difference vs control – 3.5% *Females* Difference vs control – 13.7% • 100 mg/m³ *Males* Difference vs control – 25.4% *Females* Difference vs control – 51%
Maltoni *et al*., 1977 Maltoni *et al*., 1984 (Klimisch 3) Rats	Mammary gland	600 mg/m³	*Females* Mammary tumours	Survival *Females* 59/100 vs 98/10 in the control group No data on body weight gain
Maltoni *et al*., 1977 Maltoni et al., 1984 (Klimisch 3) Mice	Kidney, Mammary gland Pulmonary system	40 mg/m³	Kidney adenocarcinomas Mammary tumours Pulmonary adenomas	100 mg/m³ was found to be the highest tolerable dose by mice
Lee *et al*., 1977 (Klimisch 3) Rats	(liver)	220 mg/m³	Non neoplastic lesions Liver: mild to markedly severe focal, disseminated vacuolisation, probably fatty change Neoplastic Haemangiosarcoma in the mesenteric lymph node or subcutaneous tissue 2/36 animals	No remarkable adverse signs One female rat terminated No deaths in the control group. Body weights of the female rats were generally less than that of the female controls after the 4th week. Body weights of the males generally less than that of the male controls after the 24th week.
Lee *et al*., 1977 (Klimisch 3) Mice	Pulmonary system Liver	220 mg/m³	Bronchioloalveolar adenoma Haemangiosarcoma of the liver in males. 3 hepatomas [hepatocellular carcinomas] 2 skin keratoacanthomas	Two males were terminated during the 9th month and one female during the 10th month (all liver tumours). Weight gains of the male and female mice comparable to those of the controls
Rampy *et al*., 1977 / Quast *et al.*, 1986 (Klimisch 3)	Mammary gland	100 mg/m³	*Females* Mammary gland adenocarcinoma	Mortality was slightly higher in the 100 mg/m³ group in the very last months of the study. For female rats

Study	Target organ	Lowest dose tumours/lesions are observed	Neoplastic and non-neoplastic incidences	Mortality and body weight changes compared to controls
Rats				at 100 mg/m³, a trend towards increased cumulative percentage of mortality was noted during the 14th to the 24th months (statistically significant at months 15, 17 and 21). Mean body weights of male rats at 100 mg/m³ significantly lower than the controls for the first 13 months of exposure and remained lower. Mean body weights of male rats at 300 mg/m³ were significantly lower from 6th to 12th months. General trend toward decreased body weights from the 17th to 24th months. Mean body weights of female rats comparable to controls or slightly higher.
Cotti *et al*., 1988	Haematopoietic system Mammary gland	400 mg/m³	Benign and malignant tumours of the mammary gland Leukaemia	Slight decrease in body weight in males and females
Dermal				
Van Duuren *et al*., 1979 (Klimisch 3) Mice			No local sarcomas, no skin papillomas When tested as initiating agent with PMA as promoter, 9 skin papillomas in 8 mice, 1 skin squamous cell carcinoma in 1 mouse	No data available

Mouse is the most sensitive species and male is the most sensitive sex for certain types of toxicity by VDC. Various studies ranging from acute to chronic toxicity confirm this observation in accordance with the metabolic pathway presented in the toxicokinetics section. However, this is not clearly established for carcinogenicity as different tumours at different doses with different latencies are observed in the different species tested.

By the oral route, the most reliable NTP (1982) study does not reveal a clear carcinogenic effect in rats and mice. However, there are several primary tumours reported in rats but the accidental deaths that occurred during week 82 of the study have an impact on statistics. Most of these tumours are statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test but become not significant when life table analyses are used. In addition, there is doubt as to whether the maximum tolerated dose (MTD) was achieved, since mortality rates and body weights of controls and dosed groups are similar. Other available studies report either only non-neoplastic lesions (Quast *et al*., 1983) or scattered incidences of tumours, but are of limited quality, in particular since the methodology followed is significantly different from the OECD TGs, short duration of exposure is applied and few details on the protocol and/or results are provided (Ponomarkov & Tomatis, 1980). Therefore, RAC considers that the available evidence is not sufficient to conclude on the absence of carcinogenic effect by the oral route of exposure.

By the inhalation route, the well-conducted NTP studies (2015) in mice and rats are relevant for the assessment of carcinogenicity. In the following tables, the incidences of tumours (both malignant and benign) per tested dose are summarised. Relevant tumours were found in both sexes in rats and mice in the absence of excessive toxicity. The incidence of malignant neoplasms and/or the combination of benign and malignant neoplasms was increased and statistically significant compared to controls in the following tumors: malignant mesothelioma, renal tubule carcinomas, adenoma, or combined, C-cell adenoma, carcinoma or combined of the thyroid gland, mononuclear cell leukaemia, haemangioma or haemangiosarcoma, hepatocellular adenoma or carcinoma. Moreover, some tumours showed reduced tumour latency, as indicated in table 21 of the CLH report. Therefore, the carcinogenic activity of VDC via inhalation can be concluded.

Table: *Tumours (malignant and benign) incidences (statistical significance* ***P<0.05 in bold****) in the NTP (2015) inhalation rat study relevant for classification*

Type of tumour	**Sex**	**Dose (mg/m³)**			
		0	**100**	**200**	**400**
Rats					
Malignant mesothelioma	Male	1/50	**12/50**	**28/50**	**23/50**
	Female	0/50	1/50	1/50	0/50
Thyroid gland adenomas/ carcinomas	Female	3/50	**10/50** (**6 carcinomas**)	9/48/	**13/50** (2 carcinomas)
Mononuclear cell leukaemia	Female	10/50	11/50	13/50	**25/50**
Renal tubule adenoma/ carcinoma	Male	3/50	4/50 (2 carcinomas)	6/49 (1 carcinoma)	2/50 (1 carcinoma)
	Female	0/50	0/50	1/49 (1 carcinoma)	0/50
Clitoral gland adenoma/ carcinoma	Female	5/47 (1 carcinoma)	8/48	3/45	9/48 (5 carcinomas)
Respiratory epithelium adenoma (nose)	Male	0/49	0/50	1/50	**4/50** (P=0.051)
	Female	0/50	0/50	0/50	1/50

Table: *Tumours (malignant and benign) incidences (statistical significance* ***P<0.05 in bold****) in the NTP (2015) inhalation mice study relevant for classification*

Type of tumour	**Sex**	**Dose (mg/m³)**			
		0	**25**	**50**	**100**
Mice					
Hepatocellular adenoma	Male	37/50	35/50	33/50	25/50
	Female	37/50	30/50	30/50	29/50
Hepatocellular carcinoma	Male	26/50	19/50	15/50	29/50
	Female	9/50	16/50	14/50	**20/50**
Hepatocholangioma	Male	1/50	2/50	2/50	3/50
	Female	0/50	1/50	1/50	2/50
Renal tubule adenoma/ carcinoma	Male	0/50	**11/50** (6 carcinomas)	**37/49 (24 carcinomas)**	**27/50 (18 carcinomas)**
	Female	0/50	0/50	0/50	1/50
Haemangioma/ Haemangiosarcoma	Female	4/50	6/50	6/50	**11/50**
Alveolar/ bronchial adenoma/ carcinoma	Female	4/50 (1 carcinoma)	5/50 (2 carcinomas)	9/50 (7 carcinomas)	7/49 (5 carcinomas)
Small intestine adenoma/ carcinoma	Male	1/50	3/50	1/50	2/50
	Female	2/50	1/50	2/50	4/50

Other studies, as shown in the table "Summary of the reported neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for the assessment of the carcinogenic properties of VDC" above, report mammary tumours, kidney adenocarcinomas, pulmonary and bronchioloalveolar adenomas, both in male and female rats and mice but can only be used as supporting evidence, as these studies suffer from several limitations. For example, studies were conducted with too short duration of exposure to study the carcinogenic potential (e.g., 52 weeks or less), or with only one dose applied or with not enough animals and in addition the results were inconsistent and/or unreliable as the tumourigenic response may have appeared only in the low dose, with no dose-related increase in tumours compared to controls etc. Nevertheless, the increase, for example, in the incidence of kidney neoplasms reported by Maltoni *et al.* (1984) is consistent with the observations made in the NTP (2015) studies in male rats and mice and increases the confidence that the kidney is a target organ of carcinogenesis of VDC. In addition, mammary tumors were also observed in other supporting studies (Maltoni *et al*., 1984; Quast *et al*., 1986; Cotti *et al*., 1988).

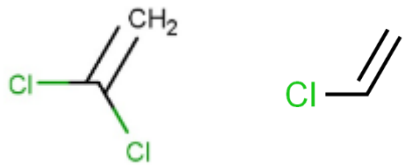
Via dermal exposure, data is limited and suffers from serious limitations, especially from the absence of information on systemic toxicity, the use of only one dose and the few study details available. No neoplasms were reported, but when tested for its initiating activity in a two-stage mouse-skin assay, VDC could be regarded as an initiating agent, as skin papillomas and skin squamous cell carcinomas were developed in mice (Van Duuren *et al*., 1979). Therefore, the carcinogenic activity of VDC via dermal exposure cannot be excluded.

In conclusion, there are several lines of evidence pointing to the carcinogenic activity of VDC. More specifically:

1. There is sufficient evidence of carcinogenic activity of VDC in male F344/N rats based on increased incidences of malignant mesotheliomas. There is also clear evidence of carcinogenic activity of VDC based on increased incidences of renal tubule adenoma and carcinoma in male B6C3F1/N mice and systemic haemangioma or haemangiosarcoma

(combined) in female B6C3F1/N mice. Supporting evidence is derived from studies showing various tumors as already explained above and shown in the preceding table, hence VDC is considered as a multisite carcinogen in both sexes of mice and rats.

2. The sufficient evidence demonstrating the carcinogenicity of VDC in experimental animals, as explained above, originates from studies using the inhalation route of exposure. However, due to insufficient and/or low-quality data, the carcinogenic effect of VDC via oral or dermal exposure cannot be ruled out, and the route of exposure will not be stated in the hazard statement.
3. RAC considers that VDC also warrants classification as a suspected mutagen, based on evidence for mutagenic activity in *in vitro* studies that include exogenous metabolic activation and in the available *in vivo* comet assay. The observation of tumors in liver and kidneys of mice and rats (and to a lesser extent in the lungs), are fully consistent with the positive results in the same tissue of the comet assay and suggest a mutagenic MoA for the tumours. VDC is metabolised to mutagenic compounds (e.g., epoxides) and there is no evidence that this (or any other) potential mechanism of carcinogenicity is not relevant to humans.
4. VDC possesses a high degree of structural similarity with vinyl chloride (see the Figure below), which has a harmonised classification (Index number 602-023-00-7) for carcinogenicity in Category 1A, H350. According to the CLP Regulation, 3.6.2.2.6, structural similarity to substance(s) for which there is good evidence of carcinogenicity may be taken into consideration when assessing the overall level of concern. Both substances undertake a similar metabolic pathway, by being metabolised by CYP2E1 to electrophilic metabolites. In the IARC assessment of VDC (IARC, 2019) it is noted that tumour induction by VDC in rodents shows many similarities to that of vinyl chloride: both compounds induced tumours in the lung, tumours of the mammary gland, and hepatic haemangiosarcomas in mice. The induction of hepatic haemangiosarcomas induced in mice has also been observed with other vinyl halides (vinyl fluoride and vinyl bromide) that are metabolised by CYP2E1 to DNA-reactive haloethylene oxide intermediates. Hepatic haemangiosarcomas are extremely rare in the general population, but significantly elevated in workers exposed to vinyl chloride.



Vinylidene chloride *Vinyl chloride*

Figure: Structural similarity of vinylidene chloride and vinyl chloride

Therefore, taking into consideration all the above, RAC agrees with the DS that a **classification as Carc. 1B; H350: May cause cancer is warranted for VDC.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance is included in Annex VI of the CLP Regulation without an aquatic hazard classification and is very volatile (vapour pressure of 66340 Pa at 20°C). The Dossier Submitter (DS) proposed to classify 1,1-dichloroethylene with Aquatic Chronic 3. The acute L/EC_{50} values were all above the cut-off of 1 mg/L for aquatic acute classification and acute classification was not warranted. The only available chronic toxicity data EC_{10} for algae was above the classification cut-off value of 1 mg/L for a not rapidly degradable substance. The surrogate system with fish LC_{50} of 107.9 mg/L did not warrant classification. Whereas the EC_{50} of 37 mg/L for *Daphnia* for a not rapidly degradable substance warranted Aquatic Chronic 3 classification.

Degradation

There was one reliable ready biodegradability study (OECD TG 301 D) available showing 0% degradation after 4 weeks. Estimations using BIOWIN (v.4.10) models also predicted the substance being not rapidly degradable.

The DS concluded that hydrolysis is not a significant degradation pathway for 1,1-dichloroethylene. In a study by Jeffers (1989) half-life of 1.2 x 10^8 years in neutral to slightly basic pH was calculated. Chloroacetylene was identified as a hydrolysis product in alkaline hydrolysis. The study did not follow standard guideline recommendations but was considered reliable with restrictions. Cline and Delfino (1987) determined half-life of 6 to 9 months (pH range from 4.5 to 8.5) and Schmidt-Bleek et *al*. (1982) estimated a DT_{50} of 2 years (pH 7).

There were no relevant water/sediment or simulation studies available.

The DS concluded that 1,1-dichloroethylene is not rapidly degradable.

Bioaccumulation

There was one reliable fish bioaccumulation study (OECD TG 305 C (1981), GLP) available for the substance. The test was suitable for volatile substances and the test substance concentrations were maintained on a constant level. Test was performed with *Cyprinus carpio* under flow-through conditions for 6 weeks. BCF values ranged from 2.5 to 6.4 and <13 at concentrations 500 and 50 µg/L, respectively.

Log Pow values from reliable sources ranged from 2.02 to 2.13 showing low potential for bioaccumulation.

The DS concluded that 1,1-dichloroethylene has a low potential for bioaccumulation.

Acute aquatic toxicity

Table: *Summary of reliable information on acute aquatic toxicity*

Method (*	Species	Test material purity	Results	Remarks	Reference
Fish					
EPA-660/3-75-009 13 days	Fathead minnow *(Pimephales promelas)*	>99.5%	96-h LC_{50} 108 mg/L	2 (reliable with restrictions)	Anonymous (1977)

flow-through			measured	Clear plastic cover over each aquarium	
Invertebrates					
OECD 202, GLP static test	*Daphnia magna*	99.95%	48-h EC_{50} 37 mg/L measured	1 (reliable) Glass flasks stoppered with PTFE bund and sealed with aluminium caps, no head space	Anonymous (2010)
Algae					
No specific guideline **72-hour static test**	*Chlamydomonas reinhardtii*	>99%	72-hr EC_{50} 9.12 mg/L measured biomass growth inhibition	2 (reliable with restrictions) adaptation for volatile compound	Brack *et al*. (1994)

(* Vapour pressure of the substance is 66340 Pa at 20°C

There was one reliable study available for each trophic level. The results were a 96-hour LC_{50} of 108 mg/L for fish, a 48-hour EC_{50} of 37 mg/L for *Daphnia magna* and a 72-hour EC_{50} of 9.12 mg/L for *Chlamydomonas reinhardtii*.

The DS also presented supporting studies that were, however, considered not reliable.

The lowest acute aquatic toxicity value was a 72-hour EC_{50} of 9.12 mg/L.

Chronic aquatic toxicity

T*able:* *Summary of reliable information on chronic aquatic toxicity*

Method (*	Species	Test material purity	Results	Remarks	Reference
No specific guideline **72-hr static test**	*Chlamydomonas reinhardtii*	>99%	72-hr EC_{10} 3.94 mg/L measured	2 (reliable with restrictions) adaptation for volatile compound	Brack *et al.* (1994)

(* Vapour pressure of the substance is 66340 Pa at 20°C

There was only one reliable study to assess chronic toxicity of 1,1-dichloroethylene. The EC_{10} for the algae *Chlamydomonas reinhardtii* was 3.94 mg/L.

The DS also presented a supporting study considered as not reliable.

There was no data available for fish and invertebrates.

The DS concluded that no chronic aquatic classification is warranted based on the algae study because the 72-hour EC_{10} of 3.94 mg/L is above the chronic classification cut-off 1 mg/L for not rapidly degradable substances (CLP Annex I Table 4.1.0 (b) (i)).

Regarding fish and invertebrates with no chronic toxicity data, according to the DS, the surrogate system for the lowest acute toxicity result, 48-hour EC_{50} of 37 mg/L for *Daphnia magna*, warrants **Aquatic Chronic 3** classification (CLP Table I Table 4.1.0 (b)(iii)).

The *Daphnia magna* study (Anonymous, 2010) follows the OECD TG 202 (GLP) and was conducted at nominal concentrations of 0, 25, 32.8, 43.2, 57.4, 76 and 100 mg/L. The study was performed using glass flasks stoppered with PTFE bungs and sealed with aluminium caps in order to avoid the loss of the test item. The test flasks were totally filled allowing no head space. The test concentrations were measured at test initiation and at the end of the static test using a validated GC-MS method. The EC_{50} values were determined based on geometric average values of initial and final measured concentrations being 0 (< LOD), 16.0, 20.5, 29.4, 36.8, 49.3 and 70.9 mg/L. In this study a LC_{50} (48 hours) of 37 mg/L was determined.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS conclusion to consider 1,1—dichloroethylene as not rapidly degradable based on:

- no degradation in a ready biodegradability study (OECD TG 301 D) in 4 weeks
- stable to hydrolysis:
    - half-lives:
        - 1.2 x 10^8 years in neutral to slightly basic pH
        - 6 to 9 months (pH range from 4.5 to 8.5)
        - 2 years (pH 7)
- no relevant water/sediment or simulation studies available.

Bioaccumulation

RAC agrees with the DS conclusion to consider 1,1-dichloroethylene having a low potential for bioaccumulation based on:

- BCF for fish below the classification cut-off 500:
    - BCF from 2.5 to 6.4 and <13 at concentrations 500 and 50 µg/L, respectively
- log Pow below the classification cut-off 4:
    - log Pow 2.02 – 2.13

Aquatic toxicity

Acute

RAC agrees with the DS conclusion that **aquatic acute classification is not warranted for 1,1-dichloroethylene**. The $L(E)C_{50}$ values for fish, invertebrates and algae were above the classification cut-off 1 mg/L (CLP Annex I Table 4.1.0 (a)).

Chronic

There is chronic toxicity data available only for algae. The 72-hour EC_{10} of 3.94 mg/L does not warrant classification (CLP Annex I Table 4.1.0 (b) (i)). There is no chronic toxicity data available

on fish and Daphnia. The surrogate system for fish 96-hour LC50 of 108 mg/L does not warrant classification.

Consequently, RAC agrees with the DS to base the chronic classification on the acute *Daphnia magna* test result 48-hour EC_{50} of 37 mg/L which **warrants classification** as **Aquatic Chronic 3** for a not rapidly degradable substance (CLP Table I Table 4.1.0 (b)(iii)).

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).